ITEMS FROM ROMANIA

AGRICULTURAL RESEARCH & DEVELOPMENT STATION — S.C.D.A.
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Dumbrava — a new winter wheat cultivar.

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Dumbrava HRWW was developed by the Agricultural Research and Development Station of Turda and was released in 2003 for its superior combination of high yield and enhanced bread-making quality. Dumbrava was selected from the cross '603106/Flamura 85//2416W2-12/Fundulea 4' using a pedigree-selection method. The single crosses '603106/Flamura 85' and '2416W2-12/Fundulea 4' were made in 1990; the final double cross was made in 1991. According to our breeding procedure (Ann Wheat Newslet **48:**113-115), the F_1 through F_2 generation were grown as bulks with assessment for agronomic types and diseases resistance. Individual heads from desirable F_2 plants were selected and planted as headrows in a nursery in 1993. Following subsequent reselection for uniformity, the resulting agronomically desirable, F_4-derived lines were evaluated in a preliminary unreplicated nursery in 1996. Thus, Dumbrava is an F_4-derived line that was tested for yield and other agronomic traits at our research station yield trials from 1997 through 1999 under the designation T95-97.

T95-97 was advanced to the Official Yield Trials at the State Institute for Variety Testing and Registration (ISTIS) in the autumn of 1999. After 3 years of evaluation (2000–02) at eight locations (24 location-years trials), the lines was registered as Dumbrava and released to growers for its good yield performance and broad adaptation to Transilvania environments and improved bread-making quality.

Dumbrava is an awned, semidwarf wheat. Juvenile growth is semierect. The foliage is green at the boot stage with a waxy bloom at anthesis. Plant height (74–92 cm) is similar to that of Fundulea 4, and 5–10 cm shorter than those of Apullum and Ariesan. Spikes are awned and middense with white glumes that are glabrous, midlong, and midwide. Kernels are red and ovate, with midsized germ; the kernel crease is midwide and middeep, with rounded cheeks. The kernel size is quite large; 1,000-kernel weight is 46–50 g and has a quite good test weight (volume weight is 74–76 kg/hectoliter).

Dumbrava is medium-late in maturity, similar to Fundulea 4 and Apullum. The winter hardiness of Dumbrava is adequate for most Transilvanian growing conditions. The cultivar has excellent straw strength, which confers good lodging resistance.

The cultiver Dumbrava is moderately resistant to yellow rust and powdery mildew, but is moderately susceptible to leaf rust. Based on artificial inoculations, Dumbrava exhibited improved resistance to FHB.

Dumbrava has shown good yield performance in most of the official test sites (ISTIS). Averaged over 3 years (2000–02) and eight locations (24 location-years), Dumbrava did not differ widely in grain yield from the highest yielding entry of the trials. Grain yield (5,832 kg/ha) was 2 % higher than that of Fundulea 4 and 7 % higher than that of Apullum. The maximum grain yield of Dumbrava (8,751 kg/ha) was obtained at the Center for Testing Varieties (CTS) Satu-Mare, in 2002. Yield stability of Dumbrava is similar to those of Fundulea 4 and Apullum.

Bread-making quality was evaluated by the ISTIS–Wheat Quality Laboratory in Bucharest. Based on 2 years (2000 and 2001) of data at five locations, Dumbrava meets domestic quality criteria for high-quality bread floor production. Compared with the quality check cultivar Apullum, Dumbrava is equal to or slightly higher in grain and flour protein content. Gluten characteristics are associated with higher SDS volume (Zeleny test). The farinographic index showed excellent dough properties (water absorption, swelling time, constancy, and elasticity) that permitted classification of Dumbrava to the B_1–A_2 quality wheat.

Breeder and foundation seed of Dumbrava, will be maintained by the Agricultural Research & Development Station, Turda.

Publications.

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ITEMS FROM THE RUSSIAN FEDERATION

AGRICULTURAL RESEARCH INSTITUTE OF THE CENTRAL REGION OF NON-CHENOZEM ZONE
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A synthetic species Triticum X duelongatum Pol. and a new line Triticum X Agropyrotriticum Cicin.

L.V. Poleva.

As a result of long-term work on hybridization of *T. durum* and *T. aestivum* with *Th. elongatum* (2n = 10x = 70), a wide diversity of hexaploid and octoploid wheat–*Agropyron* genotypes was developed, including a complete chromosomal set of durum or soft wheat and one *Th. elongatum* genome. Translocations likely occurred.

The synthetic species *T. duelongatum* Pol. (2n = 6x = 42) with the genomic formula AABBFF has been isolated. This species is self-fertilizing and high stable cytologically (Poleva and Lyubimova 1995). The species is crosses well with *T. durum, T. aestivum*, and *Triticum X Agropyrotriticum* Cicin (2n = 8x = 56). The percent of hybrid seed set increases with an increase in ploidy level, 12.5 % (with *T. durum*), 39.0 % (with *T. aestivum*), and 58.4 % (with *XAgropyrotriticum*). F_1 hybrids are fertile in the aforementioned combinations. High seed set (56 %) also has been noted in triticale (AABBRR)/*T. duelongatum* hybrids.

In the octoploid genotypes, lines have been isolated with valuable useful and practical features (high protein content, to 18 %; high spike yield, 2.3–2.5 g; and combined resistance to pathogens in the field). The lines are of interest for study as grain or hay crops and source material for wheat and triticale breeding.

The *Triticum X duelongatum* and *Triticum X Agropyrotriticum* are kept in the herbarium of Main Botanical Garden of the Russian Academy of Sciences.

References.

Poleva LV andLyubimova VF. 1995. Remote hybridization of wheat and *Elytrigia elongate* (Host.) Nevski. Bull Main Bot Garden **171**:48-60.

Selection of soft wheat lines with disomic substitutions of chromosomes of Ae. speltoides.

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Disomic substitution (2n = 42) lines of wheat were selected in a hybrid population of the cross *T. aestivum/Ae. speltoides*. Initially, flowers of a soft spring wheat were pollinated by *Ae. speltoides*. Before pollination, the pollen of *Ae. speltoides* was irradiated with gamma rays at 10 kR. Among the progeny obtained, matromorphic plants F_1M_1 with asymmetric karyotypes (2n = 49) were found. These plants had 42 chromosomes from the soft wheat parent and 14 chromosomes of *Ae. speltoides* in their somatic cells (Lapochkina et al. 1994). In subsequent hybrid generations, disomic substitution plants were found in progeny, along with 44-chromosome plants. In these plants, one of pairs of wheat homoeologous chromosomes was substituted with a pair of *Ae. speltoides* chromosomes. Plants with 44 and 42 chromosomes represent winter wheats.

Table 1. Quantitative characters in wheat–*Ae. speltoides* disomic substitution lines.

Line	Spike productivity (gm)	1,000-kernel weight (gm)	Grains/ spike	% wet protein/ grain	% leaf rust infection
Inna (standard)	2.25	52.1	43.3	12.5	15
83	1.67	34.9	47.6	14.9	0
95	1.44	36.1	39.6	12.4	0
96	1.61	36.0	44.5	13.4	0
104	1.75	31.7	54.9	15.2	0
106	1.56	32.0	48.7	14.8	0
107	1.27	31.9	39.6	16.0	0
112	1.63	34.5	47.2	15.8	0
LSD (*P=0.05*)	0.16	2.2	3.9	1.5	3

Disomic substitution lines are low in fertility and have small grain (Table 1). Many substitution lines have a higher number of grains/spike compared to the standard cultivar Inna. The advantage of disomic substitution lines over standard cultivars concerns the increased percentage of wet protein/grain and higher resistance to leaf rust. Disomic substitution lines are characterized by stable expression of traits. Flower fertility in the substitution lines does not differ from the standard cultivars of soft wheat. Disomic substitution lines have a lower winter hardiness than cultivars. These lines are tall and susceptible to lodging. During the last 8 years, no leaf rust was observed on the leaves of the disomic substitution lines. When the disomic substitution lines are crossed with soft wheat, the F_1 hybrids are resistant to the leaf rust pathogen; the resistance genes are dominant. F_2 hybrids from crosses of the disomic substitution lines and soft wheat segregate similar to monohybrids, 641 resistant:253 susceptible plants, a ratio of 2.55:1. Leaf rust-resistance genes are inherited from the *Ae. speltoides* chromosomes. In hybrid populations of the second and subsequent generations, plants not resistant to leaf rust were observed, because of gametes heterozygous for resistance or susceptibility.

Table 2. Correlation coefficients for some characteristics of *Ae. speltoides* disomic substitution lines of wheat

Characteristic	Spike productivity	Number of grains/spike	1,000-kernel weight	Wet protein content
Spike productivity	—	+0.54±0.21	+0.02±0.25	–0.21±0.24
Number of grains/spike	—	—	-0.12±0.15	+0.26±0.24
1000-kernel weight	—	—	—	–0.56±0.17
Wet protein content	—	—	—	—

The interrelationship between the expression of quantitative characters showed that spike productivity positively correlates with number of grains/spike in disomic substitution lines (Table 2). The increase of number of grains/spike results in an increase in spike productivity.

Conversely, wet protein content/grain negatively correlates with 1,000-kernel weight. A decrease in grain size results in an increase of wet protein content/grain. The other traits studied do not correlate with each other.

In disomic substitution line/soft wheat F_1 hybrids, heterosis was found for yield, spike productivity, stem length, and 1,000-kernel weight (Kyzlasov et al. 2003a). A decrease in the number of grains/spike was observed. Some disomic substitution lines contain 18.2 % wet protein/grain (Kyzlasov et al. 2003b), 59.6 % higher compared to the standard cultivar Inna (11.4 %). We found that disomic addition lines have nearly twice the amount of wet protein content/grain when compared to the standard cultivars. These disomic substitution lines can be used as parental hybrid forms in selecting soft wheats to increase of wet protein content/grain and resistance to leaf rust pathogens.

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Comparing pairs near-isogenic lines differing by alien Lr gene combinations.

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A set of NILs from the Department of Genetics at ARISER with a combination alien translocations *Lr9+Lr19*, *Lr19+Lr23*, *Lr19+Lr25*, and *Lr19+Lr26* into the genetic background of cultivars L503, Dobrynya, and line L2032 have been included in 2003 for the first time. For grain yield in 2003, NILs resistant to leaf rust are not different from cultivars and lines, except for pairs containing *Lr19+Lr23* translocations in the genetic background of the cultivar Dobrynya and *Lr9+Lr19* in the genetic background of line L2032 (Table 1).

Differences in grain yield between Dobrynya and its NILs with *Lr9+Lr19* and *Lr19+Lr23* translocation combinations were observed. The NILs had significantly lower parameters. However, between the same cultivar and NILs with *Lr19+Lr25*, no differences were observed. Nevertheless, the *Lr9+Lr19* translocation in the NILs of L2032 and also the combination of translocations *Lr19+Lr26* in the cultivars L503 and L2032 did not influence

Table 1. Infection types (IT) for reaction to local population of *Puccinia triticina,* lodging resistance, and grain yield of cultivars and isolines in 2003.

Cultivar or NIL	*Lr* genes	IT	Lodging resistance	Grain yield (t/h)
Dobrynya	*Lr19*	3	3.0	4.8
Dobrynya*4//Thatcher *Lr9*	*Lr9+Lr19*	0	3.0	4.7
Dobrynya*3//Thatcher *Lr25*	*Lr19+Lr25*	0	3.0	4.8
Dobrynya*3//Thatcher *Lr23*	*Lr19+Lr23*	0	3.0	4.2
L503*5//Thatcher *Lr26*	*Lr19*	3	3.0	4.5
L503*5//Thatcher *Lr26*	*Lr19+Lr26*	0	3.5	4.4
L2032	*Lr19*	3	3.7	4.8
L2032*4//Genaro 81	*Lr19+Lr26*	0	3.9	4.8
L2032*3//Thatcher *Lr9*	*Lr9+Lr19*	0	2.7	4.0
				F*= 5.062
				LSD = 0.4

grain test weight. For lodging resistance, the NILs in the genetic background of the cultivar Dobrynya did not differ among themselves or from Dobrynya, however, the *Lr26* translocation has increased resistance in the cultivar L503 and line L2032. At the same time, the *Lr9* translocation has lowered lodging resistance 1.2 points in line L2032.

In 2003, frequent rains during harvest caused preharvest sprouting. We evaluated for α-amylase activity for falling number. The cultivars L503 and Dobrynya have the best parameters, 443 and 400 sec, respectively. Introducing the *Lr26* translocation into the genetic background cultivar L503 significantly decreased the falling number. However, in the line L2032, this translocation (from cultivar Genaro 81) significantly increased this parameter. We have no basis to assume that the marked differences between the named lines is connected with the *Lr26* translocation or other linked genes in various genetic backgrounds. Similar results were observed for the *Lr9* translocation in the genetic background cultivar Dobrynya and line L2032.

Differences in SDS parameters were obtained only between sibs of L503 with presence and absence of translocation combination *Lr19+Lr26*. The isoline with *Lr19+Lr26* had lower SDS parameter values, differences were not observed for the other combinations.

Reaction of cultivars and T. aestivum lines to two races loose smut in Saratov.

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Two races of loose smut, race 23 (determined on a set of Russian differentials = X18, determined on Canadian differentials) and race X (unidentified), were used to assess resistance in a set of Russian cultivars and lines of T. aestivum. From Table 2, resistance to race 23 is found in cultivars with resistance genes *Ut1*, *Ut4* (suspected sources of resistance are Ostka Galicyjska, Iumillo, and Crimean), and *Ut5* (source resistance not established). The cultivar Saratovskaya 57 and line L2040 inherited resistance from a local Ukrainian cultivar. Resistance in line CI 12633 probably is from *T. timopheevii*. The source of resistance is not established in line L2358 and the cultivar Marroqui 588. Saratovskaya 60 (source of resistance Ostka Galicyjska, Crimean, and Yaroslav-emmer) and Saratovskaya 70 (hypothetical source resistance not established) are highly resistant (< 10 %).

Table 2. Reaction of cultivars and lines to races loose smut (2002–03) tested for resistance to race 18 and race X. Some loose smut-resistance genes have been identified in some of the lines.

Cultivar or line	Hypothetical source of resistance [1]	% sporulation	
		race 23	race X
Renfrew, Red Bobs, Florence/Aurore (*Ut1*)	OG	0.0	0.0
Kota (*Ut2*)	LV-RUS	75.0	65.4
Little Club (*Ut2*)	?	56.5	66.3
Carma (*Ut3*)	?	61.9	58.3
Thatcher/Regent (*Ut4*)	OG, IM, Cr	0.0	0.0
Sonop (*Ut5*)	?	0.0	0.0
Saratovskaya 36	SR	23.5	54.2
Saratovskaya 57	LV-UKR	0.0	0.0
Saratovskaya 70	?	7.7	68.2
Saratovskaya 60	OG, YE, Cr	3.2	26.2
L2040	LV-UKR	9.1	24.0
L2358	?	0.0	4.8
Marroqui 588	?	0.0	33.3
CI 12633	*T. tim*., PS	0.0	0.0

[1] OG = Ostka Galicyjska, LV–RUS = local Russian cultivar, IM = Iumillo, CR = Crimean, SR = Selivanovski Rusak, LV–UKR = local Ukrainian cultivar, YE = Yaroslav emmer, PS = Purple Straw, *T. tim*. = *T. timopheevii*, and ? = source unknown.

Race X appeared to be more virulent to many cultivars. The highest resistance was only in cultivars with genes *Ut1*, *Ut4*, and *Ut5* and also cultivar Saratovskaya 57 and lines CI 12633 and L2358.

Spike productivity of bread wheat–alien lines.

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We studied bread wheat–alien lines for parameters of spike productivity (spike length, number spikes/plant, spike weight, number of grains/spike, and grain weight/spike) for the standard bread wheat cultivars Saratovskaya 29, Saratovskaya 58, and L503 (Table 3). As a result of this analysis, we have selected two lines that surpass the productivity of the standard cultivars. The line L3266, with a 6D-chromosome substitution from 6 Ag from *Ag. intermedium*, exceeded all standard cultivars for number of spikes/ear and grains/spike. Saratovskaya 29 and Saratovskaya 58 had the largest spikes and L3266 significantly exceeded cultivar Saratovskaya 58 and was equal to Saratovskaya 29 and L503 for spike weight and grain yield/spike. Line L3262, which has the *Lr19* translocation and alien material from *T. durum* cultivar Saratovskaya zolotystaya for spike length and quantity/plant, exceeded Saratovskaya 29 and Saratovskaya 58 and was equal to L503. L3262 also exceeded Saratovskaya 58 for spike weight, quantity of grains/spike, and grain weight/spike. This line exceeded cultivars and was at a level equal to Saratovskaya 29 and L503. The lines L3269 and L3270 contain genetic material from *Ag. elongatum* and are not as good as the standard cultivars for in spike productivity except for spike length.

Table 3. Parameters of spike productivity of bread wheat–alien lines studied. The cultivar Saratovskaya is designated with an S.

		Spike character				
Cultivar, line	Pedigree	Length (cm)	No. spikes /plant	Weight (g)	No. grain /spike	Grain weight (g)
Saratovskaya 29 (S 29)		8.58	14.00	1.90	33.90	1.48
Saratovskaya 58 (S 58)		8.40	13.70	1.64	28.80	1.24
L503		9.49	15.00	1.88	33.60	1.45
L3261	Svetlana/S 55	10.60	15.40	1.85	38.60	1.41
L3262	L528*4/Saratovskaya zolotystaya	10.01	16.00	1.96	38.00	1.53
L3263	S 55*2/*T. dicoccoides**2//L528	8.63	14.70	1.57	33.50	1.17
L3264	S 66/Rodina/*Ae. speltoides*	10.24	14.50	1.52	32.70	1.07
L3265	L503*2//S 5	8.46	13.70	1.62	33.10	1.25
L3266	Rodina/Egisar 29/Moscovka 35// S29 Agro 139/L23/6R//As12	10.20	17.80	2.09	39.20	1.64
L3267	Agro 139/S29 + Agr 1	10.04	16.50	1.74	37.10	1.33
L3268	Melanopus 69/*Ag. intermedium**2//S 29	9.31	14.40	1.91	34.90	1.44
L3269	S 55/*Ag. elongatum**2//S 29	8.54	12.10	0.95	23.10	0.69
L3270	S 55/*Ag. elongatum**2//S 29	8.45	12.10	0.91	23.80	0.68
L3271	L2032/Dobrinya//L503/3/ AD *T. dicoccum*/*Ae. speltoides**5//S29	10.72	18.90	1.75	35.70	1.33
L3272	*ph 1b* CS/*Ae. umbellulata*//S 29 sk	8.62	14.50	1.90	34.40	1.52
L3273	*ph 1b* CS/*Ae. umbellulata*//S 29 sk	9.66	16.10	1.88	35.20	1.44
LSD_{05}		0.71	1.19	0.22	4.10	0.18

Black point in spring bread wheat in Saratov.

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In 2003, kernel black point was observed at 20.6 to 26.0 %. Seed was lightly infected with fungus of *Alternaria spp*. The cause of kernel black point possibly was the physiological influence of vegetative conditions. We examined the effect of black point on the germination and quality grain in popular cultivars of spring bread wheat, Belynka (white kernel), L 503 (red kernel), and Dobrynya (red kernel). Belynka is susceptible to preharvest sprouting and L 503 and Dobrynya have some tolerance. Spike sampling for germination tests began when the plant peduncle had turned yellow with no green color to the glumes (first date) and was repeated twice every third day (second and third dates). Seeds were harvested, hand-threshed, dried, and stored in refrigerator at –20°C. Germination tests were at 20±1°C in the dark on filter paper in Petri plates containing 6 ml of water.

Percent of the germination was lower for black point-infested seeds than for the control (uninfected seeds) (Table 4). These observations suggest that in 2003 black point did not contribute to preharvest sprouting. The mean 1,000 black-point kernel weight and wet-gluten content in the kernel of all three cultivars was significantly higher than the control. Mean SDS volume of the cultivars Belynka and Dobrynya for black-point kernels was lower than the control, but this difference was not significant.

Table 4. The effect of kernel black point on seed germination in three popular cultivars of spring bread wheat sampled over three dates. The first sampling date was when the peduncle had turned yellow and subsequently repeated twice every 3 days. Numbers with the same letter within a column are not significantly different at P = 0.05 level as determined by a Duncan's Multiple Range test.

		% germination at sampling date		
Cultivar	Seed source	1st	2nd	3rd
Belynka	Control	81.0 c	97.0 d	97.5 b
	Black point		89.5 c	91.0 a
L 503	Control	10.0 b	92.0 c	92.5 a
	Black point		90.0 c	90.0 a
Dobrynya	Control	0.0 a	80.0 b	93.5 ab
	Black point		68.5 a	91.0 a
Mean			86.2	92.5

Resistance of spring bread wheat to loose smut and common bunt.

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Recently in the Volga region, we have noticed an increase in loose smut and common bunt on spring bread wheat. We screened 19 cultivars and lines for resistance to loose smut and common bunt using artificial inoculation. The cultivars and breeding lines were from ARISER material. The loose smut inoculum was taken from cultivars L 505 and Saratovskaya 60. A suspension was prepared at 1.5 g spores/l water. Infection of loose smut used a medical syringe at flowering, adding to each a strict dose of inoculum. Spores of common bunt were from the susceptible cultivar Tulaikovskaya 5. For each inoculation, 1 mg spore/100 was used. Infection was directly before seeding. Disease estimates were made in field conditions.

Only two of the 19 cultivars and lines were resistant to loose smut and common bunt, L 4050-02 and L 2040. L 4050-02 has shown less than 3 % infection with common bunt and is immune to both the L 505 and Saratovskaya 60 pathotypes of loose smut. L 2040 is resistant to the pathotype collected from L 505; 11.5 % of the plants sporulating when infected with this pathotype. With the loose smut isolate from Saratovakaya 60, 8 % sporulation on plants was observed. For common bunt, the line L 2040 is moderately resistant (less than 22 % sporulation). The possible donor of resistance to these diseases in L 4050-02 is *T. turgidum* subsp. *dicoccum* and in L 2040 is *T. turgidum* subsp. *durum*.

The cultivars Lutescens 62, Yuogo-vostotchnaya 2, and line L 196 were moderately resistant to common bunt (0–10 % sporulation); line L 360-01 and cultivars L 503, L 1089, Saratovskaya 60, and Saratovskaya 29 had 11–30 %

sporulaiton. Resistance to common bunt in these cultivars and lines originated from *T. turgidum* subsps. *durum* nd *dicoccum* according their pedigrees. A group moderately resistant to loose smut included line L 894 and the cultivar Tulaikovskaya 5. The majority of the cultivars and the lines are either resistant to loose smut and susceptible to common bunt or vice versa.

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Enzyme and mycosis exhaustion in seeds of Triticum aestivum in the far eastern Russian Federation.

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The climatic conditions of the offshore and continental regions in the far-eastern Russian Federation are characterized by spring and early summer drought, summer monsoons due to the closeness of the Pacific Ocean, and high humidity. The annual precipitation in July and August makes up 65–75 % of the total annual precipitation.

For many years, research on enzyme and mycosis seed exhaustion (EMSE) has been done in western regions of the Russian Federation and the Ukraine. This research indicates that the causes of disease development were abiotic in nature, which were compounded by biotic factors including fungal diseases. Wheat cultivars show susceptibility to EMSE in high humidity and temperature conditions during the flowering and ripening stages (Alimov l988; Buryakova l974; Dunin et al. 1981; Kravchenko l98l; Shiltsova 1985).

In the far-eastern regions of the Russian Federation, scientists did not pay close attention to EMSE in spite of the spread of harmful kernel and spike diseases such as Helminthosporiosis and Fusarium wilt. The main pathogens of these diseases are *Bipolaris sorokiniana* (Saco.), *Fusarium sambucinum* Fuck., *F. semiteotum* Berk. et Rav., *F. avenaceum* (Fr.) Sacc., *F. gibbosum* App. et Wr., and *Alternaria* species. Yield losses, especially during years of epidemics, are considerable.

For a long time, the mycosis stage of the disease was studied separately from the enzymatic stage. The stage of enzyme affection of the grain was neither identified nor defined. In 200l–03, research on the spread and harmfulness of EMSE in released and perspective spring wheat oultivars from Khabarovsk selections was done at the Far Eastern Research Institute of Agriculture. This research identified biological kernel damage at milk, wax, and complete ripeness stages. Cracks in the caryopsis in the cultivar Khabarovchanka were observed. Cracks in the caryopsis and streaks and spots are typical symptoms of EMSE. The greatest number of cracks were in the pappus of kernals. The size of spots, streaks, and cracks varied from 0.5– l.2 mm (width) to 0.5–3 mm (length). Spots and streaks were on the top layer of caryopsis, some cracks were deep. Damage to the internal tissues of the grain were observed. Colonization and development of *Fusarium* and *B. sorokiniana* were noted.

In 2001, the incidence of EMSE low. During wax ripeness stage, disease development was not more than 2–3 % in some spring wheat cultivars. We noted that during our observations from 25–30 July, the first stages of the disease (enzyme), which is characterized by cracks in the caryopsis and exuding of kernal contents from the forces of osmotic pressure, were observed only in a few kernals. The typical EMSE symptoms, streaks, spots, and cracks, were not noted in the cultivar Lira during that period.

The loss of kernel contents caused by EMSE was determined in separate spikes according to the method of Dunin et al. (l981) in 2002–03. For this purpose, 40 typical spikes at milk stage were taken from different places in the plot. Spikes of the same cultivar but at different stages of ripeness (wax and complete) were selected as oontrols. The spikes were kept in waterproof bags and divided into two groups of four samples each; oontrol (dry), C1–C4, and El–E4 (experimental). In the laboratory, all kernels were immediately removed from each sample of the control group and oounted. The average humidity and l,000-kernel, dry substance weight were defined. For this purpose, the samples of group El–E4 were placed in water at a temperature of 3°C for l0 min, surplus moisture drained for 3 min, and the spikes

placed horizontally in a cell at 30°C and 95 % humidity for 48 hr. After 48 hr, all kernels from the experimental samples were removed and counted. The average humidity and l,000-kernel, dry substance weight was determined for each sample. The loss of dry substances was an index of relative cultivar stability to kernel exhaustion was defined according to the formula:

$$E\ \% = \frac{(C - E) * 100}{C}$$

The results show that the loss of dry substances in all experimental *T. aestivum* cultivars increased between the stages from milk to wax to complete ripeness (Table 1). If the loss of dry substances at milk stage in Khabarovchanka were 2.8 % in comparison with the control, the loss in Zaryanka and Lyra 98 increased from 1.8 to 19.2 % and from 5.1 to 21.1 %, respecitvely. The same results were observed for the other cultivars (Table 1). We note that dry substance losses can be partially or completely compensated for by photosynthesis at the milk or wax stages, but this process is irreversible at complete ripeness.

Table 1. Dry substance losses in *T. aestivum* cultivars in 2003 due to enzyme and mycosis seed exhaustion (EMSE).

Cultivar	Ripeness stage	Humidity (%)		Dry substance losses to the control (%)
		control	experiment	
Khabarovchanka	milk	53.5	54.8	2.8
	wax	37.4	39.8	3.8
	complete	l6.0	33.7	21.1
Zaryanka	milk	48.7	49.6	l.8
	wax	40.1	40.9	1.8
	complete	19.1	34.6	19.2
Lyra 98	milk	47.4	50.1	5.1
	wax	31.3	39.0	11.2
	complete	17.9	35.2	21.1
Erythrospermum 1/6–96	milk	52.3	5l.4	0.0
	wax	39.6	43.0	5.6
	complete	14.4	30.6	18.9
Erythrospermum 7/1	milk	58.0	55.1	0.0
	wax	39.1	41.0	3.1
	complete	16.2	34.4	21.7
Erythrospermum 13/1	milk	48.7	49.7	1.9
	wax	39.4	40.4	1.6
	complete	14.9	33.3	21.6
Erythrospermum 51/4	milk	56.6	56.4	0.0
	wax	35.8	39.6	0.9
	complete	15.0	35.4	24.0
Erythrospermum 121/3	milk	49.8	53.2	6.8
	wax	49.6	53.2	4.4
	complete	16.1	36.2	24.0
Lutescens 157/2–92	milk	46.3	53.2	4.6
	wax	35.6	39.1	5.1
	complete	17.4	33.3	19.8

In 2002, resistance to EMSE in the mature kernel after a late harvest was estimated. For this purpose, four 100-kernel samples were selected from freshly harvested grain during a period of heavy rain, prolonged dew, and fog. Both healthy, emerged, sprouted, EMSE affected, and thin kernels were marked in each sample Visually healthy caryopses accounted for 69–79 %, emerged 10–19 %, sprouted 1–7 %, visibly affected 2–8 %, and thin 2–4 % (Table 2, p. 111).

The thin-kernel symptom is not specific for EMSE. Although the thin-kernel symptom was not separately taken into account but assessed with the oomplex of conditions during ripening, it is of great diagnostic importance (Temiverkova 1996).

1n 2003 we evaluated resistance to EMSE in the cultivar Khabarovchanka during a late harvest (Table 3. p. 111). The harvest was made between 1–5 September. Visibly healthy kernels averaged 19.8 %, those effected by the first (enzyme) stage were 47.5 %, those affected by the second (mycosis) stage were 9.5 %. The total amount of kernels effected by EMSE was 74 %.

Bad weather in the summer of 2003 influenced seed quality. A late harvest led to EMSE development because a long monsoon fell during the milk and wax stages and after the crop was ripe.

The worst EMSE epidemics that can cause a 50 % yield loss happen from the combined action of both enzyme and mycosis infections. The mycosis stage causes the most harm, because semiparisitic and saprophytic fungi and bacteria, mostly species of Helminthosporium, Fusarium, Alternaria, Septoria, and Cladosporium develop more intensely during this period in the far-eastern region of the Russian Federation. Currently, one of the most important problems in T. aestivum selection is the search for sources resistant to EMSE (Shindin 2002).

Table 2. Evaluation of ripe seed of the cultivar Khabarovohanka for resistance to enzyme and mycosis seed exhaustion (EMGE) during late harvesting in 2002.

No. of samples	% visibly healthy	% emerged	% sprouted	% EMSE affected	% thin
1	79	15	4	2	0
2	69	19	4	6	2
3	79	10	7	2	2
4	74	18	1	3	4

Table 3. Evaluation of Khabarovchanka wheat seed for resistance to enzyme and mycosis seed exhaustion (EMSE) after a late harvest (commercial sowing) in 2003. The percent of sprouted kernels affected by EMSE is given in parentheses.

			% EMSE affected		
No. of samples	% visibly healthy	% sprouted	stage 1 (enzyme)	stage 2 (mycosis)	Total EMSE affected (%)
1	22	18 (13)	51	9	73
2	26	18 (16)	47	9	72
3	23	26 (24)	41	10	75
4	22	17 (15)	51	10	76
Average	23.2	19.8 (17)	47.5	9.5	74

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The impact of environment-forming plants on crop capacity and quality of wheat grain in East Siberian Predbaikaliye.

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The agroecosystems of Predbaikaliye (East Siberia, Russian Federation) are characterized by open, unstable, and low biological productivity. Turning to an environmentally friendly and biological approach to agriculture in creating highly productive agroecosystems makes extremely important the use of potential of so-called supporting environment-forming plants; both traditionally cultivated and those presently introduced in Predbaikaliye.

Evaluating the productivity of experimental crops took into account the productivity of the following environment-forming plants: goats-rue (*Galega orientalis* L.), polygonum spread (*Polygonum divaricatum* L.), hill mustard (*Bunias orientalis* L.), sainfoin (*Onobrychis arenaria* (Kit. DC), alfalfa (*Medicado sativa* L.), yellow sweet clover (*Melilotus officinalis* Desr), and the wheat crops cultivated afterwards. Experimental links to crop rotation were based on the spring wheat cultivar Angara 86, characterized by precocity, environmental stability, and high productivity. Investigations of the agronomic efficiency of agroecosystems created were conducted in 1996–2000 on the light-gray forest soils that occur frequently in Predbaikaliye. Productivity of experimental crops was compared with productivity of crop rotation link, pure fallow–wheat, which presently is used widely in regional agriculture.

Our investigations have shown that new plants of limited occurrence and those traditionally cultivated in the region have a high productivity potential (Table 1). They are capable of performing several functions in crop rotation. Their use in agricultural production contributes to strengthening of forage reserve, enrichment of soil by fresh organic substance, and intense increase of wheat productivity. As compared to crop productivity achieved in a pure fallow–wheat (3.24 t/ha) system, crop rotation links with supporting plants reached crop productivity of 5.20 to 7.55 t/ha. Productivity excess in experimental links of crop rotation as compared to control variant amounted to 1.96–4.31 t/ha in 2 years. New plants and plants of limited occurrence as predecessors after 4 years of use (2 years for yellow sweet clover) produced positive effect on soil fertility, as well as wheat productivity both in the first year and later on in the second year of use. Observations have shown that use of environment-forming plants for wheat crops in the second year upon ploughing the bed contributed to the increase of its productivity by 1.61–1.93 t/ha.

Table 1. Evaluation of agroecosystem productivity the East Siberian Predbaikaliye.

Agroecosystems (crop rotations)	Agroecosystems productivity (t/ha)			
	Preceding plants			
	Dry substance yield	Crop yield	Wheat	2-year total
Alfalfa–wheat	6.64	3.75	3.36	7.11
Sainfoin–wheat	6.32	2.17	3.12	5.29
Yellow sweet clover–wheat	6.92	2.94	2.75	5.62
Goats-rue–wheat	6.98	3.93	3.62	7.55
Hill mustard–wheat	7.62	2.53	2.67	5.20
Polygonum spread–wheat	7.94	2.64	3.21	5.85
Pure fallow–wheat	—	—	3.24	3.24

We studied the impact of new plants and plants of limited occurrence as predecessors on wheat grain quality in Angara 86. Assessing baking quality included determining protein and gluten content, assessing technological quality by determining natural mass and 1,000-kernel weight, and sowing quality, germination capacity, and energy (Table 2, p. 113)).

Protein and gluten content are key parameters reflecting quality of wheat grain, flour, and baking. Technologists believe that natural mass is an important parameter as well. Natural mass is directly related to flour yield. Grain with a low natural mass is normally frail and demonstrates low flour yield. The 1,000-kernel weight characterizes grain

Table 2. Baking, technological, and sowing parameters of wheat grain quality in crop rotations in East Siberian Predbaikaliye.

	Baking		Technological		Sowing	
Preceding plants	Protein (%)	Gluten (%)	Natural mass (g)	1,000-kernel weight (g)	Germination capacity (%)	Germination energy (%)
Alfalfa	15.4	31.8	746	30.8	93.0	92.0
Sainfoin	15.3	30.3	742	30.8	92.8	90.5
Yellow sweet clover	15.4	31.5	744	30.7	90.5	90.0
Goats-rue	15.4	32.4	747	30.8	93.0	93.0
Polygonum spread	15.0	28.2	734	30.3	91.5	91.0
Hill-mustard	15.1	29.3	742	30.4	92.0	91.0
Pure fallow	14.8	24.8	728	30.7	90.0	90.0

filling. This qualitative parameter depends on the peculiarities of the cultivar and conditions of its cultivation including preceding plant.

Major indices of seeds quality are germination and germination energy. Initial protein content of 14.8 % in wheat grain sown after pure fallow increased up to 15.1–15.4 % in wheat grain sown after traditional plants, new plants and plants of limited occurrence (the increase amounted to 10.1–10.4 % of initial content). Gluten content in wheat grain increased from 11.3–13 %. Technological and sowing qualities of grain improved. Therefore, use of potential of traditional plants, as well as new ones and those of limited occurrence, their introduction in the system of regional crop rotation contributed to the increase of wheat productivity and grain quality in Eastern Siberia.

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Pathotypes of wheat stem rust pathogenic on different plant hosts from 1996–2000.

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The mass epidemic of stem rust on wheat last year is rarely observed. The reasons for a low level of infection are resistance to the disease and the meteorological conditions during the growing season limiting development of a fungus on the host after development on the alternate host. Under favorable conditions, infections appear as separate loci on several plants. Lately, studying the composition of the populations of fungi is of decreased interest.

Annually, the urediniospores of stem rust are found in air currents throughout the Russian Federation. The aecial stage of *P. graminis* develops on different species of barberry (Elansky et al. 1998; Lekomtseva et al. 1999, 2000). Our work attempted to identify and characterize pathotypes of *P. graminis* f.sp. *tritici* on different plant–hosts in several areas of the Russian Federation, the Ukraine, and Georgia in 1996–2000.

Aecia from leaves of various barberry species were collected annually at the same sites; botanical gardens in the Moscow and Rostov (Rostov-on-Don) regions of the Russian Federation and Lvov, Ukraine. In some years, samples of wheat with stem rust were collected from plantings in the Russian Federation (Rostov and Primorski Krai regions), Ukraine (Kiev region), and Georgia (Kobuleti area). Besides isolates of stem rust from couch-grass (*Elytrigia repens*) and barley were analyzed. In rare cases, these isolated could cause a stem rust infection in wheat. Inoculation of wheat plants was done by the standard methods. To identify pathotypes (phenotypes of virulence or physiological races), 16 NILs of wheat received from the Cereal Diseases Laboratory, University of Minnesota, St. Paul, U.S., were used. Pathotypes were identified using the system of Roelfs and Martens.

The development of the aecial stage of *P. graminis* f. sp. *tritici* on various species of barberry in 1996–2000 differed according to season. As a rule, aecia developed only on separate leaves. The fungus was not found on barberry in 1997 and 2000. In collections of different species of barberries in botanical gardens, both stem rust-susceptible and resistant species are cultivated. The most significant development of stem rust on barberry was observed in 1996 and 1998, and 1999 (Table 1).

Monitoring virulence phenotypes of *P. graminis* f. sp. *tritici* in 1996–2000 on all studied species of plant–hosts indicated that the most prevalent pathotype was MKCT with virulence to genes *Sr5*, *Sr6*, *Sr7b*, *Sr8*, *Sr9e*, *Sr9g*, *Sr9d*, *Sr10*, *Sr17*, and *SrTmp* (race 34 on the Stakman scale). On wheat from Georgia, the pathotype RKCT was identified, which has virulence on *Sr5*, *Sr6*, *Sr7b*, *Sr8*, *Sr9b*, *Sr9e*, *Sr9g*, *Sr9d*, *Sr10*, *Sr17*, and *SrTmp* (race 11).

Table 1. Pathotypes of *Puccinia graminis* f. sp. *tritici* observed from 1996–2000 on leaves of various barberry species, wheat, barley, and couch-grass collected from botanical gardens or plantings in the Russian Federation (Rostov and Primorski Krai regions), Ukraine (Kiev region), and Georgia (Kobuleti area). The number of monouredinial clones selected, five from each sample, for analysis is indicated in parentheses.

Pathotype	Number of samples	Year				
		1996	1997	1998	1999	2000
Barberry						
MKCT	20 (100)	5 (25)	—	11 (55)	4 (20)	—
RKCT	—	—	—	—	1 (5)	—
Total	41 (205)					
Wheat						
MKCT	10 (50)	—	7 (35)	1 (5)	—	2 (10)
MKFT	—	—	1 (5)	—	—	—
RKCT	—	—	—	—	1 (5)	—
Total	22 (110)					
Barley						
MKCT	1 (5)	—	1 (5)	—	—	—
Total	1 (5)					
Couch-grass						
MKCT	1 (5)	—	1 (5)	—	—	—
Total	1 (5)					

In 1997 in the Moscow region, seven of 16 tester lines of wheat from the U.S. cultivated in a field for multiplication were infected with stem rust. The degree of damage reached 100 %. On wheat from the agricultural station of the Moscow University surrounding a site for multiplication of wheat lines, stem rust was absent. On these lines, pathotype MKCT also was dominant, and pathotype MKFT was identified on one of the tester lines. The presence of these infections can be explained by susceptible lines of the plant-host not of local origin.

Using the Pgt virulence system of Roelfs and Martens (1988), three pathotypes of *P. graminis* f. sp. *tritici*. were registered for the first time during 5 years on various plant–hosts in some areas of the Russian Federation, Ukraine, and Georgia. Pathotype MKCT simultaneously was found on barberry, wheat, and couch-grass. Two pathotypes of wheat stem rust were found within the European continent; MKCT in Italy and Turkey and RKCT (*Sr5*, *Sr6*, *Sr7b*, *Sr8*, *Sr9b*, *Sr9e*, *Sr9g*, *Sr9d*, *Sr10*, *Sr17*, and *SrTmp*) in Yugoslavia (Manninger 1994; Stojanovic et al. 1994; McCallum et al. 1994, 1999). We also found the

Table 2. Virulence of isolates of *Puccinia graminis* f. sp. *tritici* from various plant–hosts. Number (%) of monouredinial isolates on plant–hosts virulent to wheats with *Sr* genes.

Gene	Barberry	Wheat	Barley	Couch-grass
Sr5	100.0	100.0	100.0	100.0
Sr6	100.0	40.0	100.0	100.0
Sr7b	91.0	100.0	0.0	100.0
Sr8a	100.0	25.0	0.0	100.0
Sr9a	90.6	76.9	0.0	100.0
Sr9b	0.0	46.1	100.0	0.0
Sr9c	0.0	0.0	0.0	0.0
Sr9d	96.8	92.3	100.0	100.0
Sr9g	100.0	100.0	100.0	100.0
Sr10	100.0	61.5	0.0	100.0
Sr11	0.0	7.7	0.0	100.0
Sr17	93.7	92.3	100.0	0.0
Sr21	0.0	0.0	0.0	0.0
Sr30	0.0	0.0	0.0	0.0
Sr36	0.0	0.0	0.0	0.0
SrTmp	90.6	69.2	100.0	100.0

RKCT pathotype in the samples of wheat from Georgia. At the same time, the stem rust-resistance genes *Sr9b*, *Sr9e*, *Sr11*, *Sr21*, *Sr30*, *Sr36*, and *SrTmp* are useful against the majority of stem rust pathotypes. Thus, they are of interest for selection of wheat lines resistant to stem rust (Table 2, p. 114).

Unfavorable conditions for the development of stem rust can cause *P. graminis* f. sp. *tritici* infections on additional plant–hosts. These results can be important when epidemics of stem rust develop on wheat in favorable conditions.

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Virulence of wheat stem rust, Puccinia graminis Pers. f. sp. tritici, in the Russian Federation and Ukraine in 2001–02.

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Monitoring of virulence of the most dangerous wheat diseases is important for selection of resistance to the pathogen. In 2001 in some regions of the Russian Federation and Ukraine, a strong infection of wheat stem rust was observed. The analysis of virulence of *P. graminis* f. sp. *tritici* from some areas of the Northern Caucasus (the Rostov region), from central Russia (the Moscow region), and also from the area of the Chernobyl Atomic Power Station (the Kiev region), Ukraine were studied. In 2002, wheat stem rust development was low. However, we collected samples of the plants infected with stem rust on separate small sites within these regions. For the first time in 2002, the virulence of a wheat stem rust pathotype from western Siberia (the Tomsk region) was studied.

The definition of pathotypes of the stem rust fungus was made using Pgt-lines of wheat. In 2001, the pathotype TKNT, which is virulent to *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr21*, *Sr30*, *Sr36*, and *SrTmp* was observed in all areas (27 % of all studied isolates) and, in 2002, it was observed in central and western Siberia (6 %). The pathotype MKBT (*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9d*, *Sr9g*, *Sr10*, and *SrTmp*) was found in the Russian Federation in 2001 (9 %) and 2002 (20 %). MKBT was found in all areas of the Russian Federation (including western Siberia) and Ukraine. In 2001 in the Northern Caucasus and Ukraine, pathotypes TKNS (*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9e*, *Sr9g*, *Sr10*, *Sr21*, *Sr30*, and *Sr36*; 12 %), TKNP (*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9g*, *Sr21*, *Sr30*, *Sr36*, and *SrTmp*; 9 %) and TKPT (*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9e*, *Sr9g*, *Sr21*, *Sr30*, *Sr36*, and *SrTmp*; 7 %) were found frequently.

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Effect of light quality on the development and some physiological parameters of the durum wheat plants and calli cultivated in vitro.

Light is one of the most important factors of a plant life, not only as a source of energy for photosynthesis, light also is involved in a variety of regulatory processes of plant growth and development. Light-dependent processes do not consume much energy but are very sensitive to the light spectrum due to specific properties of some plant photoreceptors absorbing at a narrow band of photosynthetically active radiation (PAR) (Voskresenskaya 1979; Kefeli 1987; von Armin and Deng 1996). Some different types of the receptors have been described, including phytochromes (red light), cryptochromes, and phototropin (blue light). Furthermore, plants contain innumerable flavoproteins and carotenoproteins, significantly complicating the quest for the one or few that might function as blue-light receptors (Volotovsky 1987; Ahmad 1999; Lin 2000; Briggs 1999). Heliochrome, a kind of green light receptor, might be associated with phytochrome and cryptochrome (Tanada 1984). Some initial stages of plant growth (seed germination, stem and leave morphogenesis, development of the chloroplasts, and authotorophy) are tightly associated with functions of the phytochrome and a blue light receptors.

The photoregulatory reactions of intact plant tissues might be quite different from those of callus tissues, depending on the ability of calli to consume an organic carbon from the nutrition medium. Some studies on the effects of light quality on plant growth and some metabolic processes were contradictive (Tikhomirov et al. 1987; Shalaeva et al. 1991; Karnachuk and Golovatskaya 1998). Very few papers address the effects on callus tissue (Karnachuk and Gvozdeva 1998; Butenko 1964). Because callus production is essential for selection, particular attention should be paid to further investigating the effects caused by a different light qualities.

In the present study, we examined the effects of the light spectrum on growth and some photosynthetic parameters of intact shoots and callus tissues of durum wheat.

Material and methods. Seed of the durum wheat cultivar Ametist were incubated in damp, 96-well polystyrene plates subjected to continuous illumination by the blue (BL), green (GL), red (RL), or white (WL) light from fluorescent tubes (Osram, Germany) or in the darkness (D) at 25–28^{o}C. Leaf length and the dry weight of the leaves, roots, and grains were determined from day 3 to 11. Samples were fixed in a drying chamber at 105^{o}C for 15 min and at 60^{o}C for 2 h. Chlorophyll concentration in the leaf tissue was determined on days 3, 5, and 7. Chlorophyll extraction used cold 85 % ethanol (Shlyk 1971). The square of the leaf plate was determined on day 10 and the specific space density value (SSD) was calculated according to the equation: m/S (mg/cm^2) (Tooming 1977).

To induce callus formation, the upper parts of mature embryos were incubated on Murashige and Skoog (1962) medium supplemented with 2.0 mg/ml of 2.4-D. After 6 weeks, the calli were transferred to another tube with the same medium without hormone supplements to induce organogenesis. Calli were incubated in the light conditions described above for the intact shoots. Linear dimension and fresh and dry weight of the calli were measured after 6 weeks of incubation on the hormone-free medium (the end of observation).

Results and Discussion. Our study detected ambiguous effects caused by the different light spectrum in 1-week-old durum wheat seedlings (Table 1, p. 117). The maximum length of the first leaf was detected under RL; the minimum in the D group. We did not detect a depression in development caused by BL as has been reported for *A. sativa*, *Bergenia crassifolia* L. Fritsch., *Serratula coronata* L., *Lichnis chalcedonica* L., *Rhaponticum carthamoides* Willd.-Iljin (Karnachuk and Golovatskaya 1998), *Zea mays* L., *Cucumus sativus* L., and *Helianthus annuus* L. (Tikhomirov et al. 1987). On the contrary, BL stimulated shoot development compared with WL. The weight of the shoots in plants illuminated with BL was significantly larger than that of those grown under WL or in darkness, whereas significant distinctions were not detected among the RL, BL, and GL groups.

Table 1. Morphometric measurements of the shoots, roots, and leaves of 1-week durum wheat plants grown under the different light conditions. Values are averages at 95 % confidence limits.

Light	Dry weight (g) shoots	Dry weight (g) roots	Leaf length (cm)
White	0.0078 ± 0.0004	0.0045 ± 0.0002	10.31 ± 0.33
Blue	0.0106 ± 0.0008	0.0048 ± 0.0006	11.75 ± 0.35
Red	0.0098 ± 0.0008	0.0039 ± 0.0003	13.17 ± 0.30
Green	0.0094 ± 0.0004	0.0045 ± 0.0002	11.21 ± 0.28
Dark	0.0068 ± 0.0004	0.0039 ± 0.0002	7.99 ± 0.36

At the same time, the illumination variations did not alter root development. Data from the literature concerning biomass accumulation were quite different and contradictive. Tichomirov et al. (1987) showed that the biomass of cucumber and garden radish plants was maximum under BL, whereas the biomass of spring wheat was greatest under RL. Thus, the effect of different light spectra on plant biomass accumulation is still underinvestigated and, obviously, depends on many factors including plant species, exact light wavelength parameters and, probably, other conditions of the cultivation collaborating with light quality.

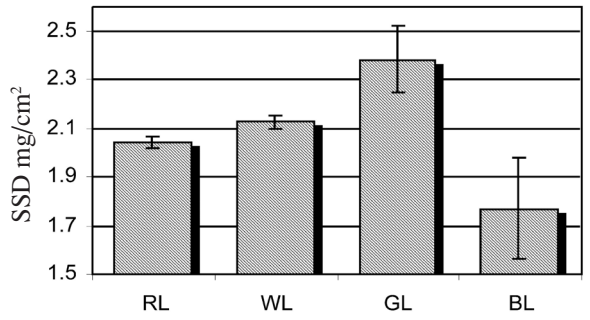

Fig. 1. The specific space density (SSD) values of the first leaves of 1-week-old durum wheat plants grown under the different light spectrum. RL = red light, WL = white light, GL = green light, and BL = blue light.

The SSD value, which represents the accumulation of dry matter per square unit, was proposed by some authors as a coefficient joining plant development with the photosynthesis processes, i.e., the more SSD detected, the greater the effect of the photosynthesis processes (Tooming 1977; Rasulov and Asrarov 1982). The SSD determined on day 10 was greatest in leaves grown under GL and, consequently, decreased in the following order: GL > WL > RL > BL (Fig. 1). The literature on this subject is limited and ambiguous. An increase in SSD under RL compared with BL was detected in the garden radish (Drozdova et al. 1987). A decrease in SSD was detected under GL in the *R. carthamoides*. In the *B. crassifolia* and *L. chalcedonica,* the SSD was greater under BL compared with RL. Finally, no dufference between BL and RL was found in *R. carthamoides* (Karnachuk and Golovatskaya 1998).

Table 2. The effect of quality of radiation on chlorophyll a + b content in first leaves of durum wheat plants (mg/g f.m.). Values are averages at a 95 % confidence limit.

Light	Day 3	Day 5	Day 7
White	0.568 ± 0.005	1.460 ± 0.003	1.585 ± 0.006
Red	0.047 ± 0.002	0.842 ± 0.005	0.820 ± 0.001
Blue	0.012 ± 0.003	0.650 ± 0.006	0.697 ± 0.014
Green	0.010 ± 0.004	0.608 ± 0.007	0.697 ± 0.006

The maximum chlorophyll concentration was detected in leaves grown under WL, followed by RL (a twofold decrease). The lowest concentration was detected under BL and GL (Table 2). The fastest chlorophyll accumulation occurred between day 3 and 5. Initially suppressed, chlorophyll accumulation increased dramatically under RL, GL, and BL compared with WL. In 1-week-old shoots grown under BL and GL, no difference in chlorophyll concentration was observed (44 % of WT).

White and red light definitely stimulated callus development (Table 3). The lowest values of both fresh and dry weight were recorded for calli grown under BL. Thus, this part of the spectrum did not facilitate development. Red light promoted formation of mostly moist calli. Monochromatic light compared with darkness in inhibited develop-

Table 3. Weight and diameter of the calli grown under the different light conditions. Values are averages with 95 % confidence limits.

Light	Weight at the end of experiment (g) Fresh	Weight at the end of experiment (g) Dry	Diameter of calli Experiment 1	Diameter of calli Experiment 2
White	0.680 ± 0.128	0.058 ± 0.008	0.84 ± 0.04	1.41 ± 0.07
Blue	0.262 ± 0.021	0.026 ± 0.004	0.71 ± 0.03	1.16 ± 0.08
Red	0.982 ± 0.276	0.047 ± 0.014	0.66 ± 0.05	1.26 ± 0.12
Dark	0.378 ± 0.072	0.038 ± 0.008	0.93 ± 0.04	1.41 ± 0.08

ment of calli grown on 2.4-D medium. After the calli were transferred to hormone-free medium, the effect vanished. Linear measures of the calli grown in the different light conditions were not significantly different at the end of observation. Similar light effects were described calli of spring wheat (Karnachuk and Gvozdeva 1998).

The current study demonstrated various effects caused by the light conditions and particularly by the light spectrum in the intact durum wheat shoots and callus tissues cultivated *in vitro*. Red and blue light, being favorable for shoot growth, inhibited development of calli. Red light also was important for chlorophyll, but it was insufficient for biosynthetic processes because they were improved almost twofold under WL. At the same time, photosynthetic processes were quite effective under GL because of improved leaf morphology, which subsequently resulted in length and weight equalization of the shoots grown under the monochromatic light.

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The effects on root system acidification and aluminum tolerance from a pretreatment of wheat seeds prior to sowing with succinic acid.

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Succinic acid increases the resistance of plants to aluminum toxicity. We investigated the influence of succinic acid on the acidification of the root system in wheat seedlings of different cultivars (exchange H^+/K^+). Our research studied the mechanism of succinic acid action. Possible components of the effect of succinic acid, including hormonal regulation, chelation of Al^{3+} to decrease in aluminum toxicity, and the influence on K streams in roots, were estimated. A high specificity of roots and leaves on succinic acid is established.

Introduction. Modern ideas about aluminum tolerance in plants assume the simultaneous action of one or more mechanisms. One effective mechanism for root stability in plants is the capability of organic acids to form chelated aluminum ions (Delhaize et al. 1993; Ma et al. 1997). The potential role of organic anion exudates in conferring resistance to Al has been evaluated. Aluminum ions impede the potassium ions in a root and effect whole-root metabolism (Poukhalskaya and Gurin 2003).

Pellet et al. (1995) found 5–9 times the output malonate becomes more active in a solution containing aluminum ions. Other organic acids, except aconite acid, did not change. However, malonate, the lowest homolog of succinic acid, is the antagonist of succinic acid in some reactions (Malygin 1995). Gassmann and Schroeder (1999) suggest that potassium and aluminum ions compete to carry ions through the plasma membrane. We investigated the influence of succinic acid on potassium metabolism, absorption, and ion exchange (H^+/K^+), assuming that potassium is transported into cells by means of a proton pump. The efficiency of these parameters was estimated by a decrease in aluminum toxicity in the roots of seedlings of three spring wheat genotypes grown in solution.

Materials and methods. We estimated the acidification activity of the root system (AARS) after treatment seeds with succinic acid (internal influence of the succinic acid on AARS) and the AARS by immersing roots in a solution of succinic acid of increasing concentration (external influence of succinic acid on AARS) and determined the role of internal succinic acid on the change in aluminum toxicity.

Estimating AARS after treatment of seed with succinic acid (internal influence of the succinic acid on AARS). Seeds of spring wheats were treated with a succinic acid solution (0.05 %) for 24 h. The seedlings were grown in 30 ml of $CaSO_4$ x 10^{-4} M (solution 1) one plant/pot. One hundred plants/genotype were grown. Two days-after-germination (20°C day/18°C night ± 2°C), the seedlings were transferred to KCl x 10^{-3} M + $CaSO_4$ x 10^{-4} M (solution 2). The change in pH in solution 2 is an integral parameter of solution acidification, because potassium and hydrogen ions are transferred (exchange H^+/K^+) through the root membrane. The pH was measured individually for each plant; root length and vegetative measurements also were made. The change in pH between solution 2 from solution 1 was calculated for each of the 100 plants. The specific acidification activity (SAARS) of the root system was calculated by dividing the AARS by the total length of root system.

Estimating AARS by immersing roots in a solution of the succinic acid of increasing concentration (external influence of the succinic acid on AARS). The same techniques as above were used in the absence of succinic acid. Succinic acid was added in a solution containing KCl x 10^{-3} M + $CaSO_4$ x 10^{-4} M at concentrations of 0.05 % and 0.1 %, respectively (solution 3). Seedlings were transferred to solution 1 for 24 hr after treatment in solution 2 and then put in solution 3. Differences in the pH of these solutions were characterized as influence of succinic acid.

The role of internal succinic acid in aluminum toxicity. Seeds of the spring wheat cultivars Priokskaya, Lada, and Ester were soaked for 30 min in H_2O_2, washed in H_2O for 40 min, and then soaked in a 0.05 % solution of succinic acid for 24 h. After that treatment, the seeds were put in a water solution in plastic pots (300 ml, 10 plants/pot) in a climate-controlled chamber maintained at 20°C day/18°C night ± 20°C. Three nutrition regimes were used: (I) H_2O + $CaSO_4$ x

10^{-4} M (control), (II) KCl x 10^{-3} M + $CaSO_4$ x 10^{-4} M, (III) KCl x 10^{-3} M + $CaSO_4$ x 10^{-4} M with Al^{3}+ ($AlCl_3$ at pH = 5.6) at a concentrations of 3 mg/l. The roots of the plants were in solutions for 18 days. Solutions were replaced every 2 days. After 18 days, the plants were weighed and growth parameters determined.

Results. Succinic acid has an effect on the root system (Table 1). Seedlings with similar root lengths and treated with succinic acid had a 1.35 times shift in solution pH (Fig. 1). The pH shift was caused the greater intensity of K+ pumps, which implies that the accumulation of succinic acid in seeds is able to influence the absorption activity (SAARS). Treatment of seeds with succinic acid treatment did not influence root length after 7 days of growth. The internal succinic acid concentration does not produce an observable change of the population into two groups.

Table 1. Influence of succinic acid on acidification activity (AARS) on the root system and the specific acidification activity (SAARS) in seedlings of spring wheat. AARS is the difference in measurements before and after salts enter the roots (ΔpH Ca^{+2}+K^{+}) and SAARS the difference in measurements divided by the total root length.

Parameter	Without succinic acid	With succinic acid
Total Root length	1204.90 ± 24.4	1204.10 ± 14.4
AARS	0.43 ± 00.064	0.64 ± 00.048
SAARS	0.0156 ± 00.009	0.0292 ± 00.004

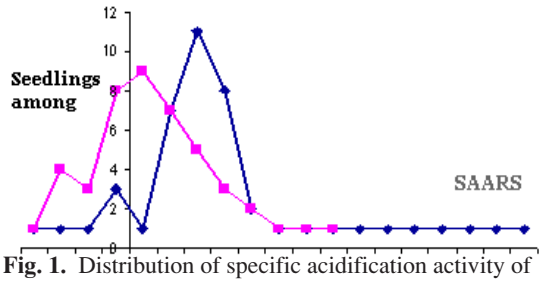

Fig. 1. Distribution of specific acidification activity of the root system (SAARS) of 100 sprouts under the influence of an succinic acid. The lines indicated the control without succinic acid (squares) and with succinic acid (diamonds).

The external succinic acid concentration is always greater than the internal concentration. The external concentration of succinic acid always caused population biotypes to disappear. In our experiments, the external concentration of succinic acid (0.1 %, 0.05 %, and 0.01 %) causes stress in the plants (Table 2). The negative influence of H^{+} ions (acidity of a solution) on root systems is known. The root system of the cultivar Priokskaya in the presence of H^{+} ions caused a decrease in root length.

Table 2. Change of root length of wheat seedlings after 9 days of growth depending on succinic acid concentration in a solution. Data is the average from three experiments.

Succinic acid concentration (%)	Change in root length	Change in seedling length
0.05	27 % decrease	change doubtful
0.10	37 % decrease	11.4 % increase
1.00	58 % decrease	23 % decrease

The amplification of the negative influence of acidity on the root system is accompanied first by the absence of an influence on vegetative parts, then active growth, and finally by a decrease in growth of the vegetative parts.

In experiments that studied the influence of the internal action of succinic acid (seed treatment) after the addition of such factors as the level of a potash nutrition and aluminum toxicity.

In the previous experiments, we showed that the root system of wheat cultivar Priokskaya reacts to succinic acid in a solution with a presowing treatment. In these experiments, isolating the action of succinic acid anions is not possible, because it is impossible to separate that action from the negative action of protons. In experiments with a longer growth period (18 days), we studied the action internal succinic acid on growth of the root system and development of plants in three cultivars of spring wheat (Priokskaya, Ester, and Lada).

We chose three genotypes in which the root system reacts differently to the addition of succinic acid at seed treatment. The negative influence of succinic acid leads to a decrease in root length in Lada and Ester. We observed a positive effect only for roots of Priokskaya. Thus, the influence of succinic acid on root growth of root of the three genotypes is not revealed. We did find that the root system of the Priokskaya genotype reacts to succinic acid (Fig. 2, p. 121). The other two genotypes did not show an increase in root length. However, changes in leaf length were unequal for the genotypes (Table 3, p. 121). Apparently, genotypes have a distinct metabolic reaction to the action of succinic

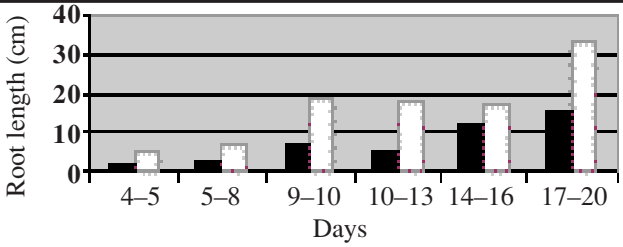

Fig. 2. Dynamics of change in root length of the wheat cultivar Priokskaya after seed treatment with (□) and without (control, ■) succinic acid.

acid. For example, comparing the weight of the root system with that of the leaf tissue of the cultivar Priokskaya, we observed an increase of 10 % with the addition of succinic acid. In the cultivar Lada, the increase was 27.1 %. The cultivar Ester has was lower for both the weight of roots leaves, resulting in a decrease of 19 %.

Similar changes are found for the influence of succinic acid on Al tolerance. Ions of aluminium (3 g $AlCl_3$/l) lead to the inhibition of root growth after immersing roots in a solution. Al toxicity decreased the growth of roots in Priokskaya by 73 %. A seed pretreatment with succinic acid caused a decrease of 69 %. For the cultivar Ester, Al toxicity in the control (without succinic acid) decreased the root length by 7 % within 12 days after germination. We did not detect a decrease in the root length when the seeds were pretreated with succinic acid. For the cultivar Lada, growth in the control plants decreased 47.8 % but no decrease was observed with a succinic acid pretreatment. The absence of decrease in growth in cultivars Ester and Lada was combined with shorter root length in plants from seed pretreated with succinic acid. The reaction to succinic acid in these genotypes was expressed in a decrease in root length. Subsequent growth in a solution containing Al ions did not have an influence. A reduction in root length was combined with an increase in root diameter, therefore, the weight of the root system was an integral parameter of the reaction of root system to Al toxicity and the factors that influence them (Table 4).

Table 3. The influence of succinic acid on changes in the length of roots and leaves in seedlings of three spring wheat genotypes. LSD_{05} = 2.4; SA = succinic acid.

	Root length (cm)		Leaf length (cm)	
Cultivar	Ca^{+2}	Ca^{+2} + SA	Ca^{+2}	Ca^{+2} + SA
Priokskaya	12.69	16.86	16.10	12.20
Lada	30.60	25.40	3.20	5.99
Ester	13.90	13.80	7.70	13.70

Table 4. Changes in the weight of the root system (g) under the influence of aluminium toxicity after a seed pretreatment with succinic acid in the presence of ions in a solution (KCl x 10^{-3} M). LSD_{05}=19.1; SA = succinic acid.

Parameter	Lada	Priokskaya	Ester
Ca^{+2} + Al	107.9	57.3	58.3
Ca^{+2} + Al + SA	61.3	85.6	55.9
Ca^{+2} + Al + K^+	101.0	82.0	59.4
Ca^{+2} + Al + SA + K^+	103.0	78.0	95.8

Potassium exchange may be a protector mechanism that determines Al tolerance by means of potash nutrition optimization and creation of competing K^+/Al^{+3} on the surface of the root system or may be a combination of mechanisms. Tolerance to aluminum in the cultivar Lada is not influenced positively by a seed pretreatment with succinic acid. Each factor separately increased stability to Al^{+3} in Priokskaya, but for Ester only an interaction of factors had an effect on increase of root weight in a stressed environment. Very different mechanisms for protection against Al ions are found in the three different genotypes. Research allows us to observe a variety of protection to Al ions in different genotypes. Studying a genotypes reaction to Al toxicity should, therefore, be multifactorial.

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The effect of nitrogen nutrition on the adaptation of spring wheat under water stress.

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In experiments with spring wheat, we studied the effects of nitrogen nutrition on the level of resistance to drought. Experiments were under controlled conditions in a phytotron. Nitrogen levels varied from insufficient to high.

Water stress was tested by discontinuing water during spike formation. Drought conditions were defined by this duration. Water stress of any duration aggravate the lack of soil nitrogen. Plant height, spike length, number of spikelets, and grain yield were reduced. Effective adaptive reactions did not develop at low levels of nitrogen, thus, the use of water was reduced. Significant depression of efficiency was marked.

High levels of nitrogen provided for high productivity, even when plants received a short-term stress. High initial nitrogen levels prior to drought influenced the growth process. A high level of nitrogen nutrition created a basis for developments of adaptable reactions and active growth during reparation period. If growth is inhibited during the period of drought, outflow of assimilates and a decrease in CO_2 absorption were observed. The intensity of photosynthesis and assimilation at the surface of leaves was reduced, but respiration level was high. Depressing physiological processes during water stress has been connected to growth activation and photosynthesis after resumption of watering.

Although the number of grains/spike was reduced, the 1,000-kernel weight increased. The high level of nitrogen nutrition appeared inefficient at strong water stress. Genotype determined the decrease in growth and water-holding capability and irreversible damage to leaf membranes. Thus, nitrogen nutrition conditions determine productivity and viability of wheat of different genotypes.

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A novel molecular marker for morphogenesis in an in vitro culture of wheat.

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The embryogenic potential of callus tissues is known to be dependent on many factors, including the functioning of embryonal antigens or morphogenetic markers, proteins associated with the embryogenic processes in an in vitro plant culture. In particular, we found that the wheat–callus–cell content of the prolific antigen of initial cells (PAI), characteristic of cereal-crop meristematic cells (Sumaroka et al. 2000), depends on the intensity of the morphogenetic processes occurring in these cells (Evseeva et al. 2002).

Using immunochemical and morphometric analyses, we studied the processes of callus formation and secondary differentiation with a genetic model including immature embryos of sister NILs (differing in the *Rht-B1c* alleles) and of the original cultivar (Saratovskaya 29) of soft spring wheat. The PAI content of immature embryos of the wheat genotypes under study was found to be the same, but it decreased in the process of formation of undifferentiated callus mass. However, the amount of PAI increased on the 21st day of callus formation (when meristematic centers were beginning to emerge in the callus tissue). Only on day 30 of callus formation were significant differences observed in

the PAI content of the genotypes under study. The PAI content of the dwarf line (*Rht-B1c* gene) was higher than that of the tall line (*Rht-B1a* gene).

Experimental estimates of the PAI content are in agreement with results of the morphometric analysis and with previous results (Djatchouk et al. 2000), attesting that PAI is associated with differentiated cell divisions in the plants. Possibly, cells marked with this antigen in the callus-tissue meristematic center perform a function similar to that of the initial cells in the whole-plant meristems. Thus, further studies of the molecular mechanisms of PAI expression in an in vitro culture may facilitate elucidating the regulatory mechanisms of cell division and of subsequent differentiation in the whole-plant apical meristems.

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Productivity of spring wheat as a function of soil temperature and nitrogen fertilizer.

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Introduction. The economic performance of agronomic plants is reflected genetically in their potential productivity, which is attained in the specific climatic and agrotechnological conditions of the region. As a rule, the production potential of crop plants is much higher than real productivity because of the influence of various abiotic and biotic factors of the external environment. The forms of N fertilizers are external factors, capable of changing the metabolism of plants because of the different modes of assimilation (Glyanko 1995; Lips 1997). In turn, N fertilizers help plants react to the adverse environments, such as low and high temperature extremes. Temperature and nitrogen, thus, are involved in attaining the potential productivity of spring wheat. We made an investigation on the influence of low (7–9°C) and high (25–30°C) soil temperatures and N- fertilizer forms (nitrate, ammonia, and urea) on spring wheat productivity.

Material and methods. The experiments were with the spring wheat cultivar Scala, cultivated in the Irkutsk area of the Russian Federation (East Siberia). Plants were grown in enameled containers containing 5 kg of air-dried soil. We used sandy soil with an insignificant level of total nitrogen (0.009 %) provided by Gelrigel medium (Grodzinsky and Grodzinsky 1973) and other nitrogen sources such as $Ca(NO_3)_2$, $(NH_4)SO_4$, and $CO(NH_2)_2$. The nutrition medium was counterbalanced with Ca and S, adding equivalent amounts $CaSO_4$ and $CaCO_3$ to the soil. To prevent nitrification in the soil, $(NH_4)_2SO_4$ and $CO(NH_2)_2$ were added along with an inhibitor of nitrification 3,5-dichlormethilpiridin (N-serve) at a level of 1.5 % of soil nitrogen. An analysis of soil in the process of nitrification has indicated that under the influence of N-serve in the soil with $N\text{-}NH^{+4}$, nitrification was noticeably hindered at optimum temperatures within 30 days. Low termperatures also had no effect on the roots. Hydrolysis of urea in the soil did not stop nitrification inhibition, but decreased at low soil temperatures.

During the summer (June–September), plants were grown in a growth chamber, five plants/container. One set of plants was grown at a soil temperature of 7–9°C (low), another at 18–22°C (control), and a third at 25–30°C (elevated). Air temperature in the chamber was not adjusted. Afternoon temperatures were within the limits of 20–27°C;

night temperatures were 10–14°C. Light exposure in the chamber was defined by natural illumination through a glass covering.

At low soil temperatures, one set of plants was grown to the 4-leaf stage (20 days after germination), the other until the beginning of milk-ripe phase (45 days). Elevated soil temperatures were given for 40 days. At the end of the experiment, the plants were transferred to conditions of the control (18–22°C) and allowed to mature. Harvest was made at full grain maturity (humidity of grain 18–20 %). The grain and above-ground parts of the plants were air dried and weighed. The biological reproducibility was ten-fold. Results are represented as the arithmetic mean with a standard error. The confidence level of the differences was evaluated by the Student's t-test. Least significant difference for comparing treatment means was at P = 0. 95 level.

Results. *Influence of low soil temperature (LST) on wheat productivity.* The influence of LTS up to the four-leaf stage did not impact grain or straw yield by any of the forms of nitrogen fertilizer (Table 1). In variants with reduced forms of nitrogen, a tendency for decreased grain and straw yield was observed in some cases, but the distinctions were inconclusive. The influence of LST causing a decrease of the total crop (grain + straw) was observed when plants received nutrition by $N–NO^{-3}$ and urea (16 and 12 %, respectively; significant at $P \geq 0.95$). In the $N–NH^{+4}$ variant, the decrease in total crop yield is doubtful.

Table 1. Productivity and structure of the spring wheat cultivar Scala depending on soil temperature and for of N fertilizer used. Soil temperature regimes are [1] 7–9 and 25–30, influence of low and high temperatures until milk-ripe stage; [2] 18–22; control conditions; and [3] 7–9, influence of low temperature until the 4-leaf stage. Items of spike structure include number of spikes, number of flowers, and number of grains.

Fertilizer	Soil temperature (°C)	Spike structure	Yield (g/container)			1,000-kernel weight (g)
			Grain	Straw	Total	
$Ca(NO)_3$	7–9 [1]	13–40–24	11.1 ± 0.75	11.1 ± 0.98	22.2 ± 1.38	33.5 ± 1.56
	18–22 [2]	15–47–32	12.2 ± 0.81	14.3 ± 1.03	26.5 ± 1.30	25.5 ± 1.43
	25–30 [1]	13–38–22	11.4 ± 0.92	15.4 ± 1.42	26.9 ± 1.68	33.8 ± 2.01
	7–9 [3]	13–36–23	12.5 ± 0.71	13.2 ± 1.45	25.7 ± 1.61	25.8 ± 2.01
$(NH_4)_2SO_4$	7–9 [1]	12–40–24	12.4 ± 0.95	11.0 ± 1.03	23.4 ± 1.40	36.2 ± 2.56
	18–22 [2]	13–38–24	12.1 ± 0.86	13.1 ± 1.26	25.2 ± 1.51	27.4 ± 1.73
	25–30 [1]	12–34–20	11.0 ± 0.75	11.1 ± 1.21	22.1 ± 1.48	31.0 ± 2.05
	7–9 [3]	12–34–26	11.4 ± 1.85	13.1 ± 1.41	24.5 ± 2.32	28.5 ± 2.55
$CO(NH_2)_2$	7–9 [1]	12–34–22	12.0 ± 1.06	12.2 ± 1.01	24.2 ± 1.46	35.0 ± 2.50
	18–22 [2]	11–33–26	13.4 ± 1.12	14.2 ± 1.35	27.6 ± 1.75	30.9 ± 1.56
	25–30 [1]	11–28–16	7.4 ± 1.32	9.7 ± 1.95	17.1 ± 2.35	27.0 ± 2.00
	7–9 [3]	12–36–23	12.0 ± 1.41	12.7 ± 1.05	24.8 ± 1.75	28.3 ± 1.73

The greatest negative impact on LST was on the straw, which decreased 20, 16, and 14 % when nitrate, ammonium, and amide were the nitrogen source, respectively (significant at $P \geq 0.95$). Nitrate and urea N sources may cause a decrease in grain yield under LST, but the results of these experiments are doubtful. Long exposure to LTS did not produce any impact on grain yield in plants with a $N–NO^{-3}$ nutrition source. The greatest negative influence of long-term exposure to LST was in the straw yield; grain yield was effected to a lesser degree. Total plant productivity under LST for 45 days was unchanged with $N–NH^{+4}$ and decreased by approximately 20 % with nitrate and urea nutrition.

Influence of elevated soil temperature (EST) on wheat productivity. Normally, plants are not tested for EST in Eastern Siberia, although physiologically and in comparison with LST it is of interest. Wheat plants do react to EST (Table 1). In plants with nitrate as a source of N nutrition, grain yield tends to decrease, but straw yield increases. Elevated soil temperatures did not produce any impact on the total productivity of plants with a nitrate source of nitrogen.

Elevated soil temperatures did produce a negative impact on wheat productivity when the source of N nutrition was $N–NH^{+4}$. This N source decreased grain yield by 9 % (not statistically significant) and straw by 15 % (significant at $P \geq 0.95$). Total productivity was lower compared to the control by 12 % (significant at $P \geq 0.95$). Surprisingly, the strong negative influence of EST on wheat productivity was exhibited with nutrition by urea. Total productivity in this case decreased by 35 % and grain and straw yields by 45 and 32 %, respectively (significant at $P \geq 0.98$ (grain yield) and $P \leq 0.99$ (straw yield)).

The impact of temperature on the structure of the main spike and 1,000-kernel weight. The long-term action of LST influenced the spike structure of the main stalk; the number of spikes, flowers, and grains was reduced under $N–NO^{-3}$ nutrition by 13, 15, and 25 %, respectively. With $N–NH^{+4}$ and urea sources of nutrition, the influence was less noticeable; the number of flowers ($N–NH^{+4}$) and grains (urea) was reduced. Short-term exposure to LST produced a significant negative impact on the spike structure. The impact of EST on spike structure is similar to that of LST; the number of spikes, flowers, and grains with all forms of nitrogen fertilizer is reduced.

The long-term influence of LST on plants using all forms of nitrogen is an increase in 1,000-kernel weight compared with the control (132, 132, and 113 % with nitrate, ammonium, and amide sources of nitrogen, respectively). The short-term action of LST has no measured influence on 1,000-kernel weight. Enhanced soil temperature increases 1,000-kernel weight with nitrate (133 %) and ammonia (113 %) but is reduced with urea (13 %).

Influence of temperature on length vegetative period of wheat. Low soil temperatures lengthen the vegetative period of wheat for 6–8 days on a short-term exposure and for 25–30 days after a long-term exposure to extreme temperatures. To the contraty, EST reduces the vegetative period of wheat 3–6 days.

Conclusions. The adverse effects of temperature on plant productivity depend on the form of N fertilizer. With $N–NO^{-3}$ nutrition, EST negatively effects grain yield and positively effects straw yield. The negative influence of EST with reduced forms of nitrogen is greatest when urea is used as a nutrition source. With long-term exposure to LST, the negative action is better shown with nutrition of an oxidizing form of nitrogen. The short-term influence LST does not give a reliable negative influence on wheat productivity of wheat, which is explained by an increase in vegetative productivity of the plants especially with an $N–NO^{-3}$ nutrition source.

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Pore permeability in the mitochondria of winter wheat.

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The participation of mitochondria in the process of programmed cell death has been reported. The main features of this process are the disruption of mitochondrial membranes, the increase of Ca^{+2} concentration in the mitochondria, permeability transition pore (PTP) opening, and the release of cytochrome c- and apoptosis-inducing factors (Jones 2000; Ferri and Kroemer 2001). Ca^{+2} ions are the most important factor for opening the PTP (Zoratti and Szabo 1995), which can be reverted by addition of different chelating agents. The opening of the PTP is inhibited by low pH, adenine nucleotides, protons, divalent cations, free-radical scavengers, polyamines, and cyclosporin A (CsA) (Zoratti and Szabo 1995; Bernardi 1996). Inhibition of PTP opening by CsA was demonstrated and shown to be mediated by binding to a family of intracellular receptors such as cyclophilins (Nicolli et al. 1996).

We know that PTP opening depends on a mitochondria-oxidizing substrate. In particular, skeletal muscle mitochondria had an increased probability of PTP opening when electron flux increases through complex I and, therefore, Fontaine and Bernardi (1999) proposed that complex I may be part of the pore complex. On the other hand, Huang et al. (2001) showed that in liver mitochondria, PTP opening is more sensitive and responsive with FADH-linked succinate.

The presence of CsA-sensitive PTP in plant mitochondria was suggested by Vianello et al. (1995) and recently was shown in potato tuber mitochondria (Arpagaus et al. 2002). At the same time, a cyclosporin A-insensitive permeability transition has been described in potato tubers and oat mitochondria (Fortes et al. 2001; Curtis and Wolpert 2002).

Because CsA is used as a diagnostic tool for the characterization of PTP in isolated mitochondria, the aim of this investigation was the influence of cyclosporin A on cold-resistant winter wheat mitochondria function and the relationships between different complexes of electron transport chain function and PTP opening.

Materials and Methods. Three-day-old, etiolated shoots of the winter wheat cultivar Zalarinka were germinated on moist paper at 26°C. The mitochondria were isolated from seedling shoots by differential centrifugation (Pobezhimova et al. 2001), and their energetic activity was studied. The isolated mitochondria were resuspended in a medium including 40 mM MOPS–KOH buffer (pH 7.4), 300 mM sucrose, 10 mM KCl, and 1 mM $MgCl_2$, with and without 5 mM EDTA. Mitochondria activity was recorded polarographically at 26°C using a closed-type platinum electrode in a 1.4 ml cell volume. The reaction mixture contained 125 mM KCl, 18 mM KH_2PO_4 (pH 7.4), 1 mM MgC_{12}, with and without 5 mM EDTA. Oxidation substrates were 10 mM malate in the presence of 10 mM glutamate, 8 mM succinate in the presence of 5 mM glutamate, and 1 mM NADH. During succinate and NADH oxidation, 3 mkM rotenone was added to the incubation medium. The concentration of cyclosporin A was 1 mkM, and the Ca^{+2} concentration of was 200 mkM. The concentration of mitochondrial protein was analyzed according to Lowry et al. (1951). Polarograms were used to calculate the rates of phosphorylative respiration (state 3), nonphosphorylative respiration (state 4), respiratory control by Chance-Williams, and the ADP:O ratio (Estabrook 1967). All the experiments were performed on 3–6 separate mitochondrial preparations.

Results and Discussion. Mitochondria are known to be one of the main sources of reactive oxygen species in the plant cell. This process can be controlled by an increase in noncoupled (Vanlerberghe and McIntosh 1997) and uncoupled (Sluse and Jarmuszkiewicz 2002) respiration. One can assume that cold resistance in winter cereals may depend on the transition of mitochondria into a state of low energy. On the other hand, PTP opening in plant mitochondria can participate in apoptotic processes.

In our work, we studied the influence of CsA on oxidation of different mitochondrial respiratory chain substrates when electrons are transferring through complexes I, II, and III. Because EDTA is a well-known chelating agent that can eliminate cations from the reaction medium, it was necessary to study the influence of CsA on mitochondrial energetic activity in the presence and absence of EDTA in all reaction media used. The influence of CsA was performed in state-4 mitochondria, because the coupling action of CsA in such mitochondria can show the existence of PTPs in plant mitochondria. The influence of the addition of CsA on mitochondrial energetic parameters was shown as a percent of state-4 respiration.

We found that at the presence of EDTA in all media with CsA added did not significant influence the state-4 respiration in mitochondria, oxidizing all substrates studied (< 9 % decrease). Because Ca^{+2} ions regulate PTP opening, we studied the influence of these ions and CsA on the function of winter wheat mitochondria in the absence of EDTA in all reaction media. We found that incubation of mitochondria with Ca^{+2} caused significant changes in their energetic activity. In malate-oxidizing mitochondria, Ca^{+2} addition caused a 46 % increase in state-4 respiration, a 48 % decrease in the RC coefficient, and an 18 % decrease in the ADP:O ratio (Table 2). In succinate-oxidizing mitochondria, this treatment caused a 19 % increase in state-4 respiration, a 28 % decrease in the RC coefficient, and a 21 % decrease of ADP:O ratio (Table 2). In NADH-oxidizing mitochondria, we detected a 22 % increase in state-4 respiration and a 33 % decrease in the RC coefficient, but we did not observed changes in the ADP:O ratio (Table 2). Such changes in mitochondrial energetic activity shows uncoupling of oxidative phosphorylating and can cause PTP opening.

Table 2. The influence of Ca^{+2} on the energetic activity of winter wheat mitochondria isolated in media lacking EDTA. 1 – mitochondria incubated without Ca^{+2}; 2 – mitochondria incubated with Ca^{+2}. Amounts shown are means ± standard error; n = 6–16; Mal, malate; Suc, succinate; Glu, glutamate.

Substrate		Rate of oxygen uptake (nMol O_2/min/mg protein) State 3	Rate of oxygen uptake (nMol O_2/min/mg protein) State 4	RC coefficient	ADP:O
Mal + Glu	1	54.4 ± 3.3	19.1 ± 2.5	2.91 ± 0.15	1.35 ± 0.22
	2	41.3 ± 3.0	27.9 ± 2.8	1.52 ± 0.12	1.11 ± 0.21
Suc + Glu	1	60.2 ± 3.1	42.1 ± 3.4	1.48 ± 0.31	0.82 ± 0.16
	2	53.8 ± 2.1	50.3 ± 2.4	1.07 ± 0.03	0.65 ± 0.19
NADH	1	79.7 ± 7.6	48.7 ± 5.9	1.72 ± 0.54	0.51 ± 0.14
	2	68.1 ± 5.6	59.3 ± 7.1	1.16 ± 0.17	0.58 ± 0.14

The absence of EDTA in the incubation media for winter wheat mitochondria, CsA addition caused a decrease in oxygen consumption in malate-, succinate-, and NADH-oxidizing mitochondria. The most significant changes were detected in mitochondria incubated with the addition of Ca^{+2}. If in the absence of Ca^{+2}, CsA addition caused a 25 % decrease in state-4 respiration in malate-oxidizing mitochondria, a 12 % decrease in state-4 respiration in succinate-

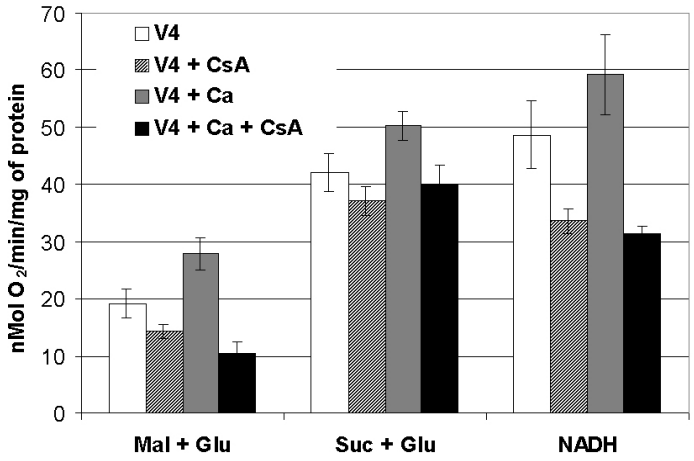

Fig. 1. The influence of cyclosporin a (CsA) addition on state-4 respiration of winter wheat mitochondria in the presence and in the absence of Ca^{+2} in incubation medium. Values are mean ± standard error, n = 3–6.

oxidizing mitochondria, and a 31 % decrease in state-4 respiration in NADH-oxidizing mitochondria, then in the presence of Ca^{+2} this treatment caused a 62 % decrease in state-4 respiration in malate-oxidizing mitochondria, a 20 % decrease in state 4 respiration in succinate-oxidizing mitochondria, and a 47 % decrease in state-4 respiration in NADH-oxidizing mitochondria (Fig. 1). We can see that the most pronounced effect of CsA addition in the absence of exogenous Ca^{+2} was detected in NADH-oxidizing mitochondria. At the same time, in the presence of Ca^{+2} in incubation medium, the most pronounced effect of CsA addition was detected in malate-oxidizing mitochondria. In succinate-oxidizing mitochondria, CsA addition caused less changes.

Therefore, our data show that CsA caused a decrease in state-4 respiration in winter wheat mitochondria. The most pronounced effect of this treatment was detected in mitochondria in the presence of Ca^{+2} ions. The influence of CsA addition is substrate-specific. Unlike animal mitochondria, the main influence of CsA in the absence of Ca^{+2} ions was detected in NADH-oxidizing mitochondria in our experiments. In our opinion, the presence of a number of rotenone-insensitive, NADH-dehydrogenases in plant mitochondria (Moller 2001) can participate in PTP formation together with complex I.

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The influence of monounsaturated fatty acids on the function of winter wheat mitochondria.

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Free fatty acids (FFA) are well-known as effective uncouplers of oxidative phosphorylation. Fatty acid-induced uncoupling occurs by different pathways: a calcium-dependent, cyclosporin A-sensitive pathway, associated with permeability transition pore (PTP) opening (Wieckowski and Wojtczak 1998; Wieckowski et al. 2000); uncoupling by interaction with some specific mitochondrial innermembrane proteins such as ADP/ATP antiporter and UCP-like proteins (Jezek 1999; Skulachev 1999); and uncoupling by function of fatty acids as low-effective protonophores (Wojtczak and Schonfeld 1993; Skulachev 1998). Free fatty acid-dependent uncoupling is inhibited by the addition of bovine serum albumin (BSA), purine nucleotides, and carboxyatractyloside, which is a specific inhibitor of ADP/ATP antiporter (Skulachev 1991). This free fatty acid-dependent uncoupling of oxidative phosphorylation functions as a cold-protective adaptation mechanism especially for lowering the content of reactive oxygen species in mitochondria (Casolo et al. 2000; Pastore et al. 2000).

Previously, cold shock was shown to cause about a twofold increase in FFA content in cereal seedling shoots (Vojnikov et al. 1983). At that time, the main FFA were unsaturated fatty acids with C16-20. In those experiments, the FFA content increased, and the uncoupling of oxidative phosphorylation in mitochondria during cold stress depended on activation of phospholipase A2 (Vojnikov et al. 1983). Ruelland et al. (2002), using Arabidopsis cell cultures, showed an increase of phospholipase C and D activity after cold treatment.

In our previous work (Grabelnych et al. 2003), we showed that linoleic acid (LA) (18:2 n-9,12) in concentrations higher than 10 mkM can not only cause uncoupling of oxidative phosphorylation in winter wheat mitochondria but that mitochondria can use this FFA as a very effective oxidation substrate. Linoleic acid concentrations higher than 50 mkM mitochondria change their metabolism to using LA as an oxidation substrate, because the rate of LA-supported respiration becomes equal to the uncoupled rate after the addition of LA respiration during malate oxidation. The LA-dependent increase in oxygen consumption is involved with the functioning of all branches of mitochondrial electron transport chain, both phosphorylative and nonphosphorylative (Grabelnych et al. 2003). At the same time, we do not know if isolated mitochondria can use other FFA that exist in plants and whose contents are increased under cold shock as oxidation substrates. The aim of this investigation was the study of possibility of a number of FFAs to be an oxidative substrate for winter wheat mitochondria.

Materials and Methods. Three-day-old etiolated shoots of the winter wheat cultivar Zalarinka were germinated on moist paper at 26°C. Mitochondria were extracted from seedling shoots by differential centrifugation (Pobezhimova et al. 2001). The isolated mitochondria were resuspended in 40 mM MOPS–KOH buffer (pH 7.4), 300 mM sucrose, 10 mM KCl, 5 mM EDTA, and 1 mM $MgCl_2$. Mitochondrial activity was recorded polarographically at 27°C using a closed-type, platinum electrode in a 1.4-ml cell (Estabrook 1967). The reaction mixture contained 125 mM KCl, 18 mM KH_2PO_4, 1 mM $MgCl_2$, and 5 mM EDTA, pH 7.4. Malate (10 mM) in the presence of glutamate (10 mM) was used as oxidation substrates. The concentrations of used monounsaturated fatty acid (erucic (22:1 n-9), oleic (18:1 n-9), and its isomer petroselinic (18:1 n-12), were 0.056 mkM–10 mM. In the first set of experiments, we added different amounts of FFAs to malate-oxidizing mitochondria in state 4. In the second set of experiments, we added different amounts of FFAs to mitochondria in a polarograph cell without adding another oxidation substrate. Polarograms were used to calculate the rates of phosphorylative respiration (state 3), nonphosphorylative respiration (state 4), respiration controlled by Chance-Williams (RC), and the ADP:O ratio (Estabrook 1967). The concentration of mitochondrial protein was analyzed

according to Lowry et al. (1951). All the experiments were performed on three separate mitochondrial preparations. The data obtained were analyzed statistically and arithmetic means and standard errors determined.

Results and Discussion. The amount of total FFA in winter wheat mitochondria is about 15 ng/mg of mitochondrial protein (0.056 mkM) and increases to ~40 ng/mg (0.15 mkM) after short-term cold shock (Vojnikov et al. 1983). In our experiments, we used physiological concentrations of FFA and higher concentrations (1 mkM–10 mM). We studied uncoupling activity and the possibility of an oxidative substrate of monounsaturated FFA that exist in plants, oleic acid, petrozelinic acid, and erucic acid.

We found that in addition to state-4 mitochondria, physiological and even higher (up to 5 mkM) concentrations of oleic acid (18:1 n–9) did not cause any changes in the rate of oxygen consumption (Fig. 2A, 1). Concentrations higher than 5 mkM caused an increase of oxygen consumption with a maximum (65 % increase as compared with the variant without oleic acid) at 30 mkM of oleic acid added (Fig. 2A, 1). Further increasing the oleic acid concentration (to 70 mkM) caused a less significant increase in oxygen consumption than when 30 mkM added. A very high concentration of oleic acid added to state-4 mitochondria (from 100 mkM up to 5 mM) did not influence on oxygen consumption (Fig. 2A, 1). The study of a possibility of winter wheat mitochondria use oleic acid as an oxidation substrate showed that all concentration of this FFA added to mitochondria did not caused any oxygen consumption by mitochondria (Fig. 2A, 2).

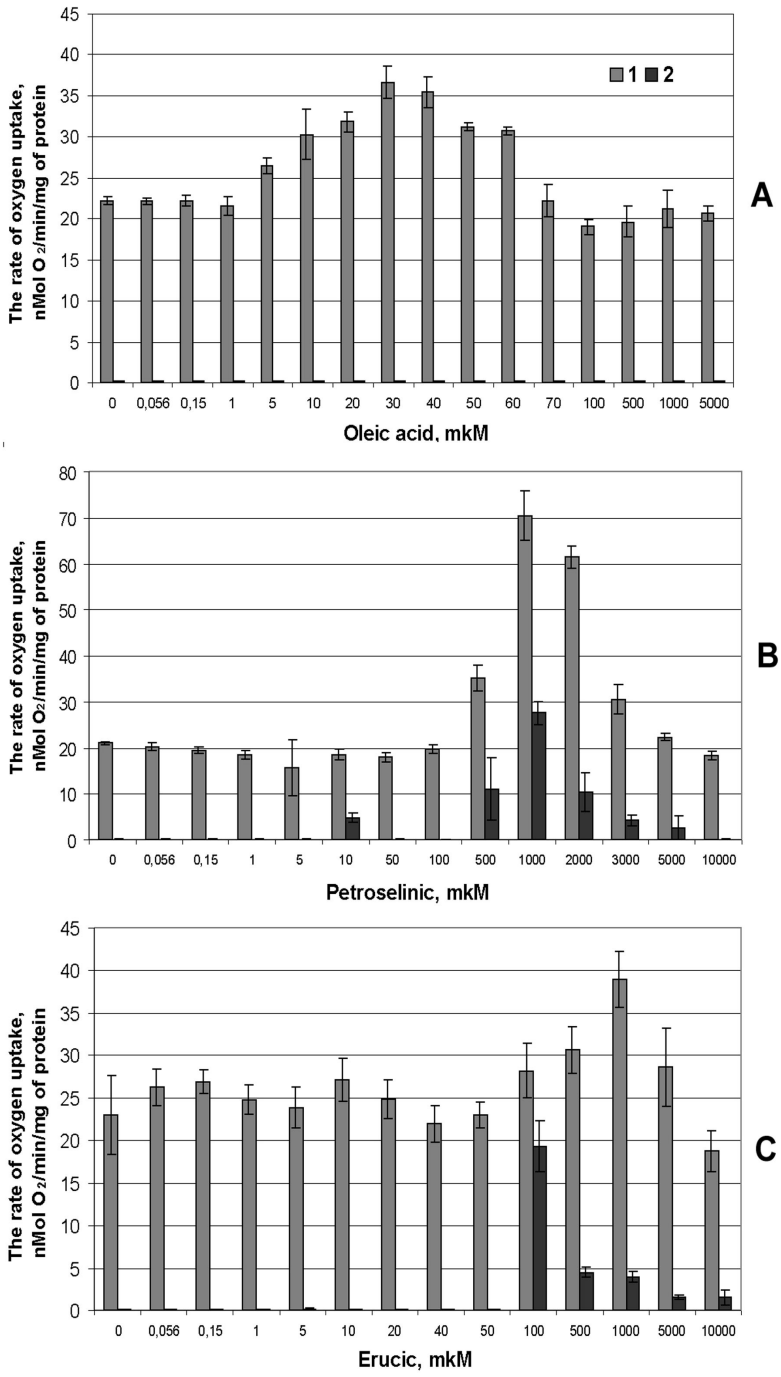

Fig. 2. Oxygen consumption in state-4, winter wheat mitochondria oxidizing 10 mM malate + 10 mM glutamate in the presence of free fatty acids (FFA) (1) and oxygen consumption of mitochondria oxidizing FFA as the only oxidation substrate (2).
A. In the presence of oleic acid; mean + standard error, n = 3–6.
B. In the presence of petroselinic acid; mean + standard error, n = 3–6.
C. In the presence of erucic acid; mean + standard error, n = 3–6.

Studying the uncoupling activity of the oleic acid isomer petroselinic acid (18:1 n–12) showed that added concentrations up to 100 mkM did not influence mitochondrial energetic activity (Fig. 2B, 1). Concentrations from 500 mkM to 2 mM caused an increase in oxygen consumption with a maximum at 1 mM (3.4-fold increase) (Fig. 2B, 1). Concentrations higher than 5 mM did not influence mitochondrial oxygen consumption. Assuming that mitochondria may use petroselinic acid as an oxidation substrate was positive (Fig. 2B, 2). The maximum petroselinic acid oxidation-depen-

dent oxygen consumption was detected at the same concentration as for FFA added when the maximum uncoupling effect was detected, 1 mM (Fig. 2B, 1, 2). At this concentration, we detected an even higher oxygen consumption than for state-4 malate-oxidizing mitochondria.

The uncoupling activity of erucic acid (22:1 n–9) showed a slight (but not statistically significant) increase in mitochondrial oxygen consumption even when physiological concentrations were added (Fig. 2C, 1). A statistically significant increase in uncoupled respiration was detected only when concentrations higher than 100 mkM of erucic acid were added to state-4 mitochondria. The maximum increase of oxygen consumption (about 63 %) was detected when 1 mM of FFA was added. Further increases in FFA concentration caused a decrease of its uncoupling effect (Fig. 2C, 1). Studying the possibility of mitochondria to use erucic acid as an oxidation substrate showed that winter wheat mitochondria can oxidize this FFA at concentrations between 100 mkM and 1 mM of FFA added. The most pronounced effect was detected at concentration 100 mkM. Further increases in the concentration of FFA added did not cause a significant increase of oxygen consumption. Therefore, erucic acid can be oxidized by winter wheat mitochondria.

As shown previously, linoleic acid can be oxidized very effectively by winter wheat mitochondria at concentrations of FFA added higher than 50 mkM but its uncoupling effect was detected at concentrations higher than 10 mkM (Grabelnych et al. 2003). It is interesting to note that uncoupling effect of oleic acid was very similar to its of linoleic acid but they are different in their possibility to be an oxidation substrate – if mitochondria can very effectively oxidize linoleic acid they cannot oxidize oleic acid. On the other hand, petroselinic and erucic acids were similar in their uncoupling action and in their possibility to be an oxidation substrate.

Based on the data from present and previous work, we can divide all FFA studied into two variants: 1. FFA that can only uncouple oxidative phosphorylation but can not be used as oxidation substrate by mitochondria and 2. FFA that can both uncouple oxidative phosphorylation and be used as oxidation substrate by mitochondria (Table 3). In this case, exogenous FFA are translocated into mitochondria through inner membranes cause cotransport of protons and, therefore, uncoupling of oxidative phosphorylation. Later, they do not move by assistance of a UCP protein into the mitochondrial intramembrane space according to the FFA-cycling mechanism hypothesis (Jezek et al. 1997) but enter the cycle of FFA β-oxidation and become oxidation substrates. Thus, unsaturated free fatty acids in winter wheat mitochondria can not only play the role of uncouplers, but also can be the only oxidation substrate for them.

Table 3. The possibility of free fatty acids causing uncoupling of oxidative phosphorylation and their ability to be an oxidation substrate in winter wheat mitochondria.

	Function	
Fatty acid	Uncoupling	Substrate oxidizing
Oleic	+	—
Erucic	+	+
Petroselinic	+	+
Linoleic	+	+

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Cold resistance in spring wheat as a function of nitrogen form.

A.K. Glyanko, N.V. Mironova, and G.G. Vasilieva.

Introduction. Cold resistance implies a plants ability to grow at suboptimal temperatures (Drozdov et al. 1984; Korovin 1984; Rodchenko et al. 1988). S pring wheat is a cold-resistant plant, so low positive temperatures within hardening range, even acting for a long time, do not bring about lethal outcome although they certainly produce a negative impact on the productivity (Korovin 1984; Glyanko et al. 2004). The cold resistance of different plant varieties of moderate climates is genetically predetermined. However, most of these plants possess minimal resistance at low temperatures and other factors optimal for growth and development. Maximum resistance in these plants indicates some unfavorable factors, for example, at hardening temperatures (Glyanko 1995). This idea is in agreement with the hypothesis of Drozdov et al. (1984) regarding the fact that natural temperatures fall into zones within which a plant demonstrates different levels of resistance within the limits genetically conditioned for a certain variety. Thermal hardening of plants by low, positive temperatures increases their resistance to extreme factors (i.e., frosts) in many plants from moderate climates including spring wheat.

Nature may condition plants to intensify their resistance depending on climatic factors. In eastern Siberia, the crop variety phenotype is adjusted for growth in temperatures that sharply change during the day. In these conditions, varieties may demonstrate more intense growth processes compared to southern varieties. Plants of the latter type, grown at low positive temperatures, reduce growth and intensity of metabolism, whereas varieties of Siberian selection in these conditions are less inhibited in growth and metabolism.

Thus, variety specificity of plants with cold resistance at low but not damaging temperatures becomes obvious. Presumably, cold-resistant varieties, with compared with those with less cold resistance, possess a stronger ability for adaptation to temperature, which allows them to reduce vital functions to a lesser extent. Nevertheless, different cultivars will show different abilities to resist cold at damaging (lethal) and nondamaging temperatures. We distinguish between resistance to low, positive nondamaging temperatures and resistance to damaging low temperatures or frosts. In the first case, plants of a resistant variety or hybrid show a more intense metabolism and growth. In the second case, plants are characterized by less intense growth and metabolic processes. The hypothesis on the protective role of a reduction of exchange process intensity in the cells towards damaging factors, which was advanced by Melekhov (1983), is particularly interesting in this light.

Based on this information and results of experiments on the impact of different nitrogen forms on metabolic processes in spring wheat (Glyanko et al. 2002a; Glyanko et al. 2003), we investigated cold resistance of seedlings of the spring wheat cultivar Skala with nutrition by different forms of nitrogen (NO^{-3} and NH^{+4}).

Material and Methods. Spring wheat plants of the cultivar Skala were grown in growth chambers of the Siberian phytotron (Irkutsk) in enamel containers. Sandy soil with a low total nitrogen concentration (0.009 %) was used as a substrate. A nutrient mixture (Thomas et al. (1978) with various nitrogen sources ($Ca(NO_3)_2$, $NH_4(SO_4)_2$) was used in the soil. Other experimental conditions, without nitrogen and with zeolite saturated with ammonium, also were used. The amount of $N–NH^{+4}$ adsorbed by zeolite from the $NH_4(SO_4)_2$ solution were equal to 448 mg/container. Zeolite was used to prevent the possible toxic impact of NH^{+4} by gradual release of adsorbed nitrogen cation into soil solution (Ando et al. 1988). Upon emergence of sprouts at 20°C, the seedlings were transferred to different temperature conditions for the remainder of the experiment: variant 1, with a 24 hours of low air temperature at 10°C; variant II, 20°C daily air temperature and 10°C night temperatures (control); and variant III, 20°C daily air temperature, 10°C night temperature plus low soil temperature (11 ± 1°C) for 24 hours.

Illumination in the chambers was provided by DRL-700 lamps. Infrared radiation was impeded by a water screen. The photoperiod was 16 day/8 night hours. Wheat seedlings were hardened (acclimated) to low temperature at the 3-leaf phase at a temperature of 10°C day and 5°C night for 5 days with artificial illumination (12 Kl). Control plants were grown at 20°C during the day and 10°C at night.

The linear growth of the plants was determined with auxanographies, which allow monitoring the growth of the topmost leaf during 24 hours (Shevelukha 1977). Protein nitrogen content was determined by the micromethod of Keldal (Ermakov et al. 1987). The tests were repeated 5–10 times. The results are represented as mean ± standard error.

Results and Discussion. Data representing average growth at different stages of plant development (2-, 3-, and 4-leaf stage) showed that the impact of 24-hour low temperatures on the plants results in a more intense inhibition of linear growth of the seedlings supplied with $N–NO^{-3}$ (by 21–25 %) compared to plants grown on reduced nitrogen (Table 4). The difference is statistically valid at $P > 0.99$. When compared to variant II (20°C day/10°C night), inhibition amounted to 63 and 49 % for plants grown on nitrate and ammonium nitrogen sources, respectively. Consequently, in comparison with the control, growth inhibition is most pronounced in plants when nitrate nutrition was used.

In the control variant II, the rate of plants growth was approximately the same at all nitrogen sources. However, in variant III, where all the plants were affected not only by low air night temperature but also by low soil temperature, growth decreased by 21 % only in the treatment with nitrate. Difference statistically significant at $P > 0.95$. Apparently, the difference was due to an intense decrease in plant growth at night when the impact of low air and soil temperatures overlapped. Therefore, wheat seedlings supplied $N–NO^{-3}$ are more sensitive to low positive temperature, which also is confirmed by data on the accumulation of dry substance by wheat seedlings. The concentration of dry substance decreases under the 24-hour influence of low temperature with a nitrate source of nitrogen (by 37 % compared to the plants absorbing $N–NH^{+4}$) (Table 5).

This data may be interpreted based on the concept of cold resistance understood as a plants ability not only to survive at low temperature, but to maintain high growth rate and productivity. Consequently, the physiological state of the plants supplied reduced form of mineral nitrogen allows these plants to be more cold resistant (as compared to the

Table 4. Rate of growth in the spring wheat cultivar Scala at different temperature regimes depending on nitrogen form, mm/h. The growth rate is the average growth value per 24 hours in different growth phases. Variant 1, 24 hours of low air temperature at 10°C; Variant II, 20°C daily air temperature and 10°C night temperatures (control); and Variant III, 20°C daily air temperature, 10°C night temperature plus low soil temperature (11 ± 1°C) for 24 hours.

Nitrogen form	Variant I	Variant II	Variant III
Nitrate	0.81 ± 0.05	2.18 ± 0.11	1.73 ± 0.10
Ammonium	1.05 ± 0.05	2.04 ± 0.06	2.02 ± 0.07
Zeolite + ammonium	1.08 ± 0.04	2.15 ± 0.08	2.15 ± 0.04
No nitrogen	0.93 ± 0.17	2.52 ± 0.22	2.29 ± 0.14

Table 5. Accumulation of dry substance in seedlings of the spring wheat Scala (surface part of the plant) at different growth temperatures (4-leaf stage), g/plant. The growth rate is the average growth value per 24 hours in different growth phases. Variant 1, 24 hours of low air temperature at 10°C; Variant II, 20°C daily air temperature and 10°C night temperatures (control); and Variant III, 20°C daily air temperature, 10°C night temperature plus low soil temperature (11 ± 1°C) for 24 hours.

Nitrogen form	Variant I	Variant II	Variant III
Nitrate	0.290 ± 0.02	0.146 ± 0.05	0.168 ± 0.01
Ammonium	0.462 ± 0.02	0.170 ± 0.01	0.197 ± 0.01
Zeolite + ammonium	0.380 ± 0.02	0.190 ± 0.02	0.210 ± 0.03
No nitrogen	0.201 ± 0.01	0.142 ± 0.01	0.175 ± 0.02

plants supplied with N–NO^{+3}). Using nitrate nitrogen for wheat nutrition is likely to ensure better cold hardening of plants. In this case, plants will switch off the implementation of productivity and switch on mechanisms that ensure survival in extreme cold conditions (Glyanko and Vasilieva 2002b). Because linear growth of plants is accepted as a criterion of cold resistance, the role of growth in thermal resistance of the plants needs comment. The role of growth processes in a plants resistance has consensus of opinion. Pollock (1990) reported on the high negative correlation between growth intensity of a number of plants and their resistance to freezing. On the other hand, Rodchenko et al. (1988) found a certain level of growth processes is an indispensable condition for increasing cold resistance of maize root cells. We believe that the different opinions regarding the role of growth in thermal resistance are due to the existence of different resistance types. In our case, this is connected with impact of low positive temperature and frosts on wheat plants. We believe that growth processes have either a positive or a negative connection with resistance depending on the type and degree of the external factor. If resistance is associated with the possibility of growth and development in the current environmental conditions, which being external still allow the plant to grow within its genotypic limits, the cultivar with the more intense growth processes and metabolism should be considered cold resistant. Under the impact of sudden damaging factors, e.g., frost, plants able to minimize vital processes will suffer the least from the destructive influence of the external factor.

Growth and metabolism are interrelated vital processes. Inhibition of growth processes brings about restructuring of metabolism. However, depending on the type of influence on the plant (extreme temperatures, physiologically active substances, or mineral nutrition), the degree of suppression or stimulation of growth processes should be different. At the same time, growth is undoubtedly under genetic control. Growth, as a polygenic feature, may be expressed at reproductive or by an increase in resistance. The former is connected with high growth intensity; the latter with little growth. Both high and low rates of growth processes are likely to be under genetic control, different growth rates of plants absorbing oxidized or reduced forms of nitrogen are most likely conditioned by various metabolic restructuring processes in the cells at unfavorable temperature. The essence of an organism's phenotypic changes is at the posttranslational level (Drozdov et al. 1984). According to Lips (1997), different growth rates in plants absorbing oxidized or reduced forms of nitrogen under external factors is associated with the disturbance of potassium–malate shunt. Our data demonstrate that this may cause a reduction in the amount of water amount available to the cells, a shift of hormonal balance towards accumulation of abscisic acid, and restructuring of protein exchange (Glyanko 1995).

Opinions regarding the role of nitrogen exchange in plant growth processes at low temperature differ (Theodorides and Person 1982; Alekhina and Klyuikova 1986; Tormanova et al. 1991). From our data, plant growth at low positive temperature is connected with intake and assimilation of nitrogen. Affecting growth and metabolism processes they may alter wheat resistance to low environmental temperature at the phenotypic level. The influence of nitrogen as a nutrition source can be seen from the data of Tables 4 and 5, where plant growth on a medium without nitrogen differs from those supplemented with nitrogen. To confirm this fact, we tested hardened spring wheat seedlings with different types of nitrogen nutrition. Taking into account the numerous data on the role of easily soluble proteins in the formation of cold resistance, we used this parameter to assess the resistance of wheat seedlings to low hardening temperatures by different forms of nitrogen nutrition. Our results indicate that cold hardening increases the concentration of both soluble (in 0.1 M Tris-buffer, pH 7.7), and insoluble protein (Table 6). However, differences between nitrate and ammonium nutrition exist. Nitrate cold hardening contributes to an increase in the

Table 6. The impact of cold acclimation on protein nitrogen content in leaves of the spring wheat cultivar Scala depending on the form of nitrogen nutrition used. ESP = easily soluble protein; NS = nonsoluble protein.

Nitrogen form	Leaf position	Protein nitrogen concentration mg/g dry tissue		NS:ESP ratio
		ESP	NS	
Acclimated plants.				
Nitrate	Bottom	5.0 ± 0.07	18.1 ± 0.31	3.6
	Middle	5.5 ± 0.10	16.5 ± 0.32	3.0
	Top	7.1 ± 0.47	27.2 ± 0.30	3.9
Ammonium	Bottom	4.5 ± 0.33	10.1 ± 0.23	2.3
	Middle	5.1 ± 0.13	7.6 ± 0.45	1.5
	Top	8.4 ± 0.26	11.6 ± 0.42	1.4
Nonacclimated plants.				
Nitrate	Bottom	4.4 ± 0.39	11.8 ± 0.47	2.7
	Middle	5.8 ± 0.43	16.6 ± 0.30	2.9
	Top	4.8 ± 0.14	21.3 ± 0.26	4.4
Ammonium	Bottom	-	-	-
	Middle	6.3 ± 0.29	17.7 ± 0.18	2.8
	Top	6.8 ± 0.10	16.3 ± 0.14	2.4

concentration of easily soluble nitrogen in bottom and top leaves, which is particularly pronounced (by 146 %) in the upper growing leaf. In the middle leaf, the concentration of easily soluble protein does not change. During hardening, nitrate-nourished plants significantly increase the content of insoluble protein in the bottom and top leaves.

In ammonium-nourished plants, cold hardening contributes to the increase of easily soluble protein concentration only in the top leaf (by 123 %) and sharply decreases the concentration of insoluble protein; the latter apparently favors protein destruction.

The ratio between insoluble and soluble proteins in unhardened nitrate-nourished plants increases from the bottom to the top leaf; the situation is reversed in ammonium-nourished plants. Cold hardening in nitrate-nourished plants results in stabilizing this ratio for all the leaves. In ammonium-nourished plants, cold hardening brings about a sharp decrease from the bottom to the top leaf.

Thus, cold hardening changes the direction of protein synthesis in wheat seedlings and, depending on nitrogen form, the character of these changes varies: nitrate nutrition provokes increase of synthesis of both easily soluble and structural proteins, ammoniium nutrition only of soluble proteins. According to Brown and Bixby (1973), the insoluble protein fraction, largely associated with membranes, is responsible for the initial stages of increase in cold tolerance, whereas maximum resistance is associated with the combined action of soluble and insoluble proteins. Temperature hardening is known to lead to synthesis of so-called stress proteins in plants (Voinikov 1989; Thomashov 1999). Our data indicate that some changes in the spectrum of proteins synthesized occur in the course of cold hardening depending on the form of nitrogen used for plant nutrition. Therefore, we favor the idea of an unequal impact of nitrate and ammonium nitrogen on metabolism in spring wheat which, in turn, affects growth processes and resistance of the plants to low temperature.

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Transgenic wheat with the gene ugt/iaglu from Zea mays generated by the biolistic techniques.

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The use of genetic engineering methodology to generate new varieties of agricultural plants with known traits is very desirable. Improvement of crops by the genetic engineering will achieve faster results than other methods of traditional breeding. *Agrobacterium*-mediated plant transformation is the most often used method for the gene transfer, but *Agrobacterium* infection is not suitable for monocots and some legumes. Biolistic techniques have been developed in order to transform these recalcitrant plants species. Biolistics is based on force of a powder or gas gun or other commercial devices. Particle bombardment or biolistics has been used to transform a large number of different plant species as well some animals, fungi, and bacteria (Finer et al. 2000).

The *ugt/iaglu* gene isolated from *Z. mays* already has been used for the transformation of tomato, potato, aspen, and some other plant species as a target gene that stimulates growth (Rekoslavskaya et al. 1999). UDPG-transferase appears to control the amount of the growth hormone indoleacetic acid in plants (Szerszen et al. 1994). In maize, the pathway to the sugar conjugates of IAA begins with the synthesis of 1-O-β-D-indol-3-ylacetyl-glucose (IAGlu) from uridine 5'-diphosphate-glucose (UDPG) and IAA, catalyzed by the enzyme IAGlu synthetase (UDPG:indol-3-ylacetyl)-β-D-glucosyl transferase, IAA + UDPG = IAGlu + UDP. IAGlu is an acyl alkyl acetal and its energy unfavorable synthesis is followed by an energy favorable transacylation of IAA from IAGlu to myo-inositol in maize. The other gene used in the study was *acb* isolated from *A. thaliana*. The ACBP protein participates in the organization of membranes by moving acyl-CoA esters inside cells.

Our goal was to generate transgenic spring wheat plants that express UDPG-transferase encoded by the *ugt/iaglu* gene from maize. The *acb* gene was used as an additional target gene. Transformation was done with a gene gun of original design by Salyaev et al. (2001).

Materials and Methods. The spring wheat cultivars Scala and Tulunskaya 12 were used. Immature embryos of 26–30-day-old plants were used as target explants for gene gun transfer of DNA. Morphogenic calli were generated from immature embryos of Scala.

Direct delivery of gene constructions by gene gun. To deliver genetic cassettes to wheat embryos, a gene gun of special design was developed on the basis of a pneumatic gun by shooting microprojectiles. This approach makes it possible to introduce genetic vectors into cells applied on the surface of tungsten microprojectiles of 1.6 μ placed on a teflon macroprojectile with a diameter of 4.5 mm. Fresh, isolated immature embryos were used for transformation by performance according to the procedure of Salyaev et al. (2001). After bombardment, transformed and nontransformed embryos were placed on P AGAR with the addition of 0.125 M mannitol and 0.125 M sorbitol. The next day, P AGARs with samples were transferred to an MS medium supplemented with 100 mg/l kanamycin for selection of transgenic shoots. PCR and Southern hybridization were according to standard procedures (Sambrook et al. 1989) described in detail in Rekoslavskaya et al. (2001) and Salyaev et al. (2001).

UDPG-transferase was extracted from plant tissue and purified from the supernatant, and activity determined according to Rekoslavskaya et al. (2001) and Salyaev et al. (2001). The amount of IAA in the plant material was determined with an HPLC according to Rekoslavskaya et al. (2001). The activity of marker enzyme ß-glucuronidase (GUS) encoded by the reporter gene *gus* was determined by standard methods (Jefferson 1987).

Results and Discussion. ***Direct delivery of gene constructions by gene gun.*** To study the expression of the selective gene *nptII*, nontransformed and transformed regenerants obtained from wheat embryos were placed on MS agar with 100 mg/l kanamycin. The effect of 100 mg/l kanamycin on the growth of biolistically transformed spring wheat with genes *nptII* and *ugt* is shown in Fig. 3 (p. 136). The growth of wheat plantlets was sharply different: transgenic plants grew faster and taller, nontransformed plants were chlorotic, small, and later became dry.

The expression of the reporter gene *gus* was studied in transformed and nontransformed embryos by determining the activity of β-glucuronidase with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (XGlc) as a substrate. Blue spots were observed in the region of apical meristems (Fig. 4) in embryos incubated with XGlc 2–3 days after bombardment. The spots were maintained for 36 days of subculture, indicating the expression of the DNA delivered via particle bombardment into target tissues.

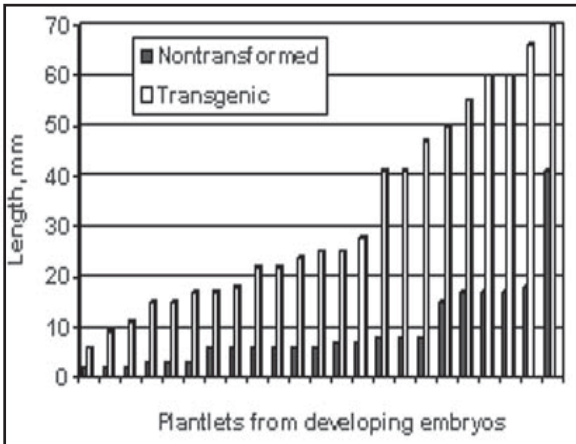

Fig. 3. The growth of transformed and nontransformed spring wheat in the presence of 100 mg/l of kanamycin.

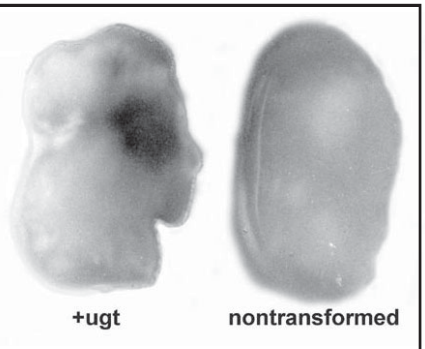

Fig. 4. Transformed (+*ugt*) and nontransformed wheat embryos after incubation in a solution of XGlc.

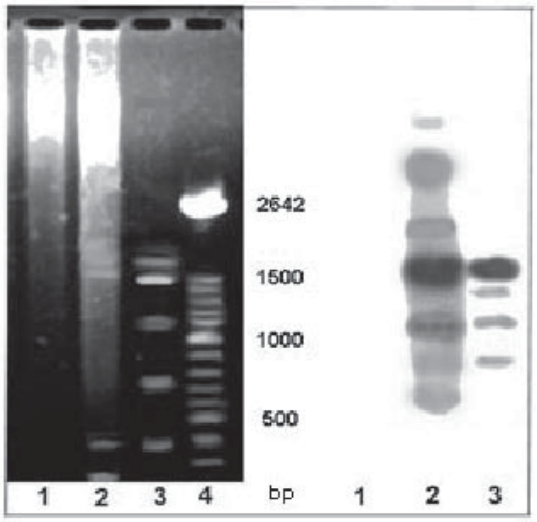

Fig. 5. PCR (left) and Southern hybridization (right) of wheat DNA isolated from transformed and nontransformed wheat embryos. Lane 1, nontransformed embryos; lane 2, transformed embryos; lane 3, the *ugt* gene in pBluescript vector; and lane 4, DNA molecular markers.

PCR with primers to the *ugt* gene revealed the homology of DNA from transformed wheat to the *ugt* gene from corn (Rekoslavskaya et al. 2001; Salyaev et al. 2001). No band of the appropriate size was observed in genomic DNA of nontransformed wheat. Southern hybridization performed on wheat DNA (Salyaev et al. 2001) confirmed the homology of the *ugt* gene from corn found in genomic DNA of transformed wheat embryos (Fig. 5).

Nontransformed and transgenic plantlets were placed in keramsite growing medium (Fig. 6) and grown until spikes developed. The growth activity of the transgenic wheat was higher in comparison to nontransformed plants (Table 7, p. 137). The height; leaf and stem number; and leaf, stem, and root mass are all higher in the transgenic plants with the *ugt* gene (Table 7). The mass of transgenic wheat plants with only the *ugt* gene was 37.9 g; the mass averaged 45.3 g with both the *acb* and *ugt* genes. The mass of one average nontransformed plants was about 2 times less, 20.3 g.

We decided to analyze the auxin status to determine the activity of UDPG-transferase and the IAA content in wheat plants. In transformed wheat there were found the higher specific activity of the target enzyme UDPG-transferase (Table 8, p. 137) and the increase in IAA content almost in all parts of transgenic wheat in comparison to nontransformed one (Table 9, p. 137).

Conclusion. We achieved the integration and expression of the gene ugt in plants of spring wheat by means of biolistic methods. The integration of the *acb* gene was determined with PCR (data not shown). Some positive effects on the growth of transformed wheat plants with both the *acb* and *ugt* genes in keramsite beds

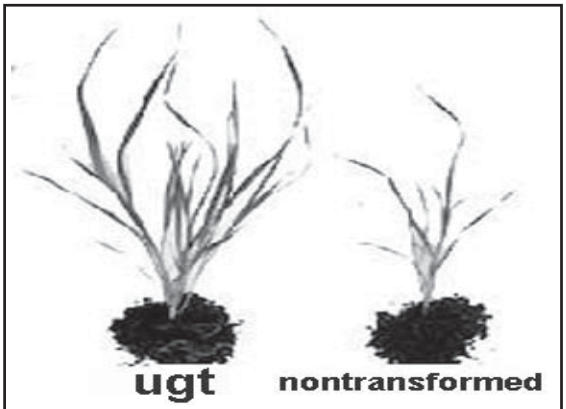

Fig. 6. The view of shoots obtained from transformed and nontransformed embryos grown in keramsite.

Table 7. The comparison of growth in transformed and nontransformed wheat plants during cultivation in keramsite beds.

Variant	Height (cm)	Number of leaves	Number of stems	Leaf mass (g)	Stem mass (g)	Root mass (g)
acb + *ugt*	48 ± 13	41.0 ± 5.2	12.0 ± 3.7	10.5 ± 3.5	23.5 ± 7.5	11.3 ± 1.6
ugt	32 ± 2	32.5 ± 10.6	10.5 ± 3.9	9.7 ± 4.9	19.3 ± 4.2	8.9 ±2.4
Nontransformed	28 ± 4	18.3 ± 4.1	5.4 ± 0.9	6.3 ± 0.5	9.7 ± 1.7	4.3 ± 0.9

according to the morphometric analyses were probably because of better adaptability. The good expression of the selective gene *nptII* and the reporter gene *gus* were revealed in transgenic wheat of both cultivars of spring wheat. We consider the biolistic method of delivering genes into target tissue was successful. With expression of the *ugt* gene, a higher activity of UDPG-transferase was determined in transgenic wheat in the cultivar Tulunskaya 12. This activity correlated with the higher contents of IAA almost in all parts of transgenic Tulunskaya 12 wheat. Transgenic wheat plants of both cultivars have an increase in growth in comparison to nontransformed wheat plants, which correlated with the highest auxin status in transgenic wheat plants. Microprojectile bombardment can be used to obtain transgenic wheat the maize *ugt* gene with the addition of the *acb* gene from *A. thaliana*.

Table 8. Specific activity of UDPG-transferase from the supernatant fraction isolated from nontransformed and transgenic wheat plants.

Variant	IAA glucose (nmol/mg protein/h)
Nontransformed	18.8 ± 5.8
acb + *ugt* genes	38.7 ± 4.4

Table 9. IAA content in nontransformed and transgenic wheat (nmol/organ).

Organ	Nontransformed	*acb* + *ugt*
Leaves	1.0 ± 0.1	3.0 ± 0.3
Stems	6.7 ± 0.1	19.9 ± 2.6
Shoots	0.5 ± 0.2	2.0 ± 0.1
Roots	0.9 ± 0.1	2.9 ± 0.2
Spikes	0.1 ± 0.0	0.3 ± 0.0

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Subunits of functional glutenin as structural elements of gluten complex proteins.

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Native wheat glutenin is a complex set of biochemically diverse polypeptides connected by different forces of protein-protein interactions. Identifying peculiarities of the genetically determined composition of glutenin functional subfractions is of interest becasue of their immediate participation in the formation of a gluten-protein complex that is responsible for the baking properties of flour.

High-molecular glutenin subunits of the majority of industrial wheat cultivars of many countries have been well studied. Important conclusions have been made regarding the participation of these structural elements in the formation of the complicated, multicomponent protein gluten complex responsible for dough and bread quality. Glutenin consists of at least 15–17 subunits with different biochemical natures (molecular mass, amino acid composition, and primary and secondary structure), associated into a single permolecular protein complex via intermolecular, disulfide bonds stabilizing a 3-dimensional, structural gluten matrix. The number of these bonds is a genotypically determined trait with a genetically specific character and determined by the physical properties of gluten and rheological characteristics of the dough (Trufanov 1994).

With the aim of finding favorable alleles, we studied the impact of individual chromosomes of homoeologous groups 1 and 6 that control storage proteins synthesis in wheat lines with intervarietal substitutions based on contrasting baking qualities cultivars on quantitative content of HMW-glutenin subunits in principal functional glutenin subfractions.

Materials and methods. Soft wheat lines with intervarietal substitutions for chromosomes 1A, 1B, 1D, 6A, 6B, and 6D of the cultivar Novosibirskaya 67 (N67), a donor cultivar that is a strong wheat, and the high-protein recipient cultivar Diamant 1 (Dm) with low technological quality were used for the investigation (Obukhova et al. 1997; Maystrenko et al. 1993).

Glutenin subfractions (GN) were obtained from freshly ground flour after removal of albumins, globulins, and gliadins by successive extraction with 0.05 m acetic acid (subfraction GN-1), 4 m urea (subfraction GN-2), and 4 m urea in the presence of 2-mercaptoethanol (subfraction GN-3) (Trufanov 1994). Protein fractions were dialyzed against 0.01 M acetic acid and lyophilized. Subunit composition in the GN-1, GN-2, and GN-3 subfractions was studied after SDS-electrophoresis in a 9 % polyacrylamide gel according to Laemmli (1970). Evaluation of the quantitative content of PAGE zones in the glutenin subfractions of each substituted line was performed by densitometric analysis of electrophoregrams (Fig. 7). The quantitative content of five HMW-glutenin subunits was calculated with the help of a computer program. The results were expressed in relative units and in percent to recipient cultivar Dm.

Results and Discussion. From the data, we concluded that the donor cultivar N67 exceeds the recipient cultivar Dm in relative content groups of glutenin subunits GS 2 and GS 3 in easily soluble fraction of GN-1 glutenin (by 10–12 %), in the sparingly soluble subfraction GN-2 in GS 4 and GS 5 (by 13 and 45 %, respectively), and in the insoluble (without restoration of SS bonds) subfraction GN-3 in GS 1 and GS 2 (by 25 and 10 %, respectively) (Fig. 8, p. 139). Simulta-

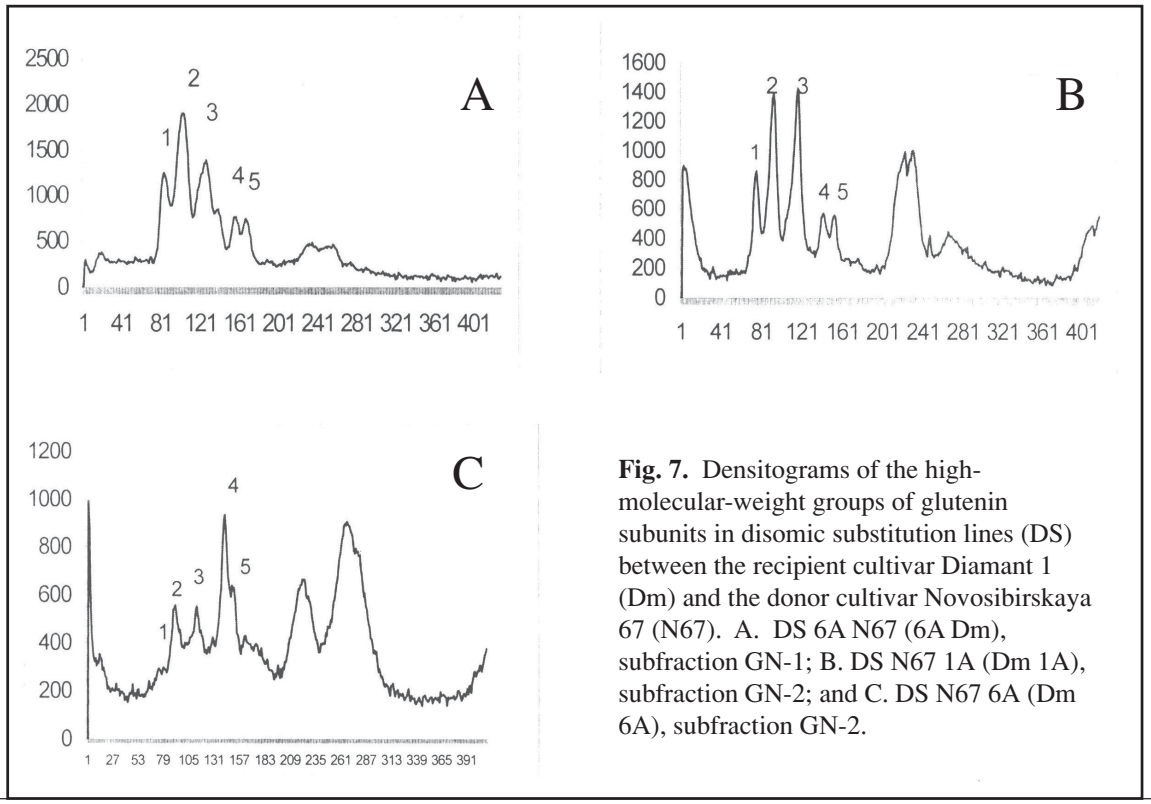

Fig. 7. Densitograms of the high-molecular-weight groups of glutenin subunits in disomic substitution lines (DS) between the recipient cultivar Diamant 1 (Dm) and the donor cultivar Novosibirskaya 67 (N67). A. DS 6A N67 (6A Dm), subfraction GN-1; B. DS N67 1A (Dm 1A), subfraction GN-2; and C. DS N67 6A (Dm 6A), subfraction GN-2.

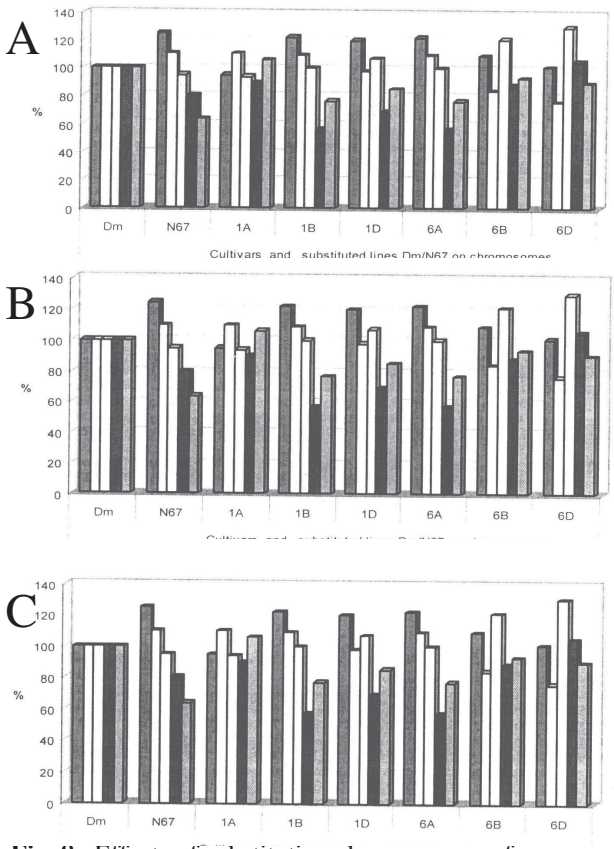

Fig. 8. Effects of substituting chromosomes of homoeologous groups 1 and 6 on the relative content of high-molecular-weight glutenin functional fractions in disomic substitution lines between the cultivars Diamant 1 (Dm) with Novosibirskaya 67 (N67). A. subfraction GN-1, B. subfraction GN-2, and C. subfraction GN-3. Columns are recipient line Dm; donor line N67; 1A, DS N67 1A (Dm 1A); 1B, DS N67 1B (Dm 1B); 1D, DS N67 1D (Dm 1D); 6A, DS N67 6A (Dm 6A); 6B, DS N67 6B (Dm 6B); and 6D, DS N67 6D (Dm 6D).

neously, the donor cultivar N67 is inferior to the recipient Dm in GN-1 content in GS 1 and GS 4 (by 11 and 20 %, respectively), GN-2 content in GS1 and GS3 (by 17 and 7 %, respectively), and GN-3 in GS 3, GS 4, and GS 5 (by 5, 20, and 36 %, respectively).

Obukhova et al. (1997) and Maystrenko et al. (1993) have shown that the subunits 1 and 2 are controlled by chromosomes 1A, 1B, and 1D in Dm and N67, whereas GS 3 contains three components controlled by chromosomes 1B and 1D. One of the components of the GS 3 belonging to N67 is controlled by chromosome 1B, one belongs to the cultivar Dm and also is controlled by 1B, and a third GS 3 component, common for both cultivars, is controlled by chromosome 1D. Several researchers (Obukhova et al. 1997; Maystrenko et al.1993; Payne 1979; Payne 1997) assumed that chromosomes of different homoeologous groups control the compositionally complicated GS 4 and GS 5 (Fig. 9, p. 140).

Compared to the recipient cultivar Dm, GS 1 content prevails in the GN-1 subfraction in substitution lines of chromosomes 1A, 6A, and 6D, in subfraction GN-2 in the line DS N67 1B (Dm 1B), and in the subfraction GN-3 in lines DS N67 1B (Dm 1B), DS N67 1D (Dm 1D), DS N67 6A (Dm6A), and DS N67 6B (Dm 6B). GS 2 prevails in DS N67 6A (Dm 6A) in subfraction GN-1, in all substituted lines of the subfraction GN-2, and in DS N67 1A (Dm 1A), DS N67 1B (Dm 1B), and DS N67 6A (Dm 6A) in subfraction GN-3.

GS 3 content was higher only in the DS N67 6A (Dm 6A) line in subfraction GN-1 and in the lines DS N67 6B (Dm 6B) and DS N67 6D (Dm 6D). GS 4 content was higher in all the lines of subfractions GN-1 and GN-3. GS 5 only was highest in subfraction GN-1 in substituted lines with chromosomes 1D and 6A and was slightly higher in DS N67 1A (Dm 1A) in subfraction GN-3.

Positive influences of glutenin on subunit content in the recipient cultivar Dm were to various degrees caused by all the chromosomes of the donor cultivar N67. Chromosome 6A caused a considerable effect. The substitution of chromosome 6A resulted in an increased synthesis of all subunits in the soluble subfraction GN-1, two of those in the sparingly soluble subfraction GN-2, and two in the insoluble fraction GN-3. Extraction of the insoluble GN-3 fraction is only possible after complete restoration of inter- and intramolecular SS links that stabilize the gluten structural matrix. Simultaneously, the content of individual glutenin subunits in the substitution lines was lower than in the recipient, particularly that of GS 3, 4, and 5.

Differences in solubility of subfractions GN-1, GN-2, and GN-3 are known to be associated with the density of their spacial structure, which determines the ability of these proteins to form the permolecular protein associates characteristic of gluten (Trufanov 1994). Formation of protein glutenin macroassociations in the grains in vivo happens with the participation of various intermolecular forces, ion-electrostatic interactions, conditioned by acid and base amino acids (AA); hydrophobic contacts (hydrophobic AA); and SS links (cystine). Consequently, the combined impact of nonvalent and covalent forces are determined by the quantity and biochemical properties of individual polypeptides (subunits), primarily by the content and location in polypeptide chains of reactive SH groups that are able to form intermolecular SS bonds. The folding of proteins of various origins and the chaperon-dependent assembling of protein macroassociations in the cell in vivo are known to happen in cotranslation and/or posttranslation periods. Folding and assembling are

catalyzed by the system of SH/SS-metabolism enzymes, in particular, by protein disulfide isomerase (Chi-Chen Yong 1998; Marusich 1998; Fisher 1998) responsible for formation, splitting, and isomerization of SS bonds in the proteins. The significant changes observed in the substitution lines in the quantitative content and proportion of HMW-glutenin subunits of functional glutenin fractions of contrasting in the technological properties of wheat cultivars may play an important role in the formation (assembling) and stabilization of functionally important glutenin complexes as a structural basis of gluten.

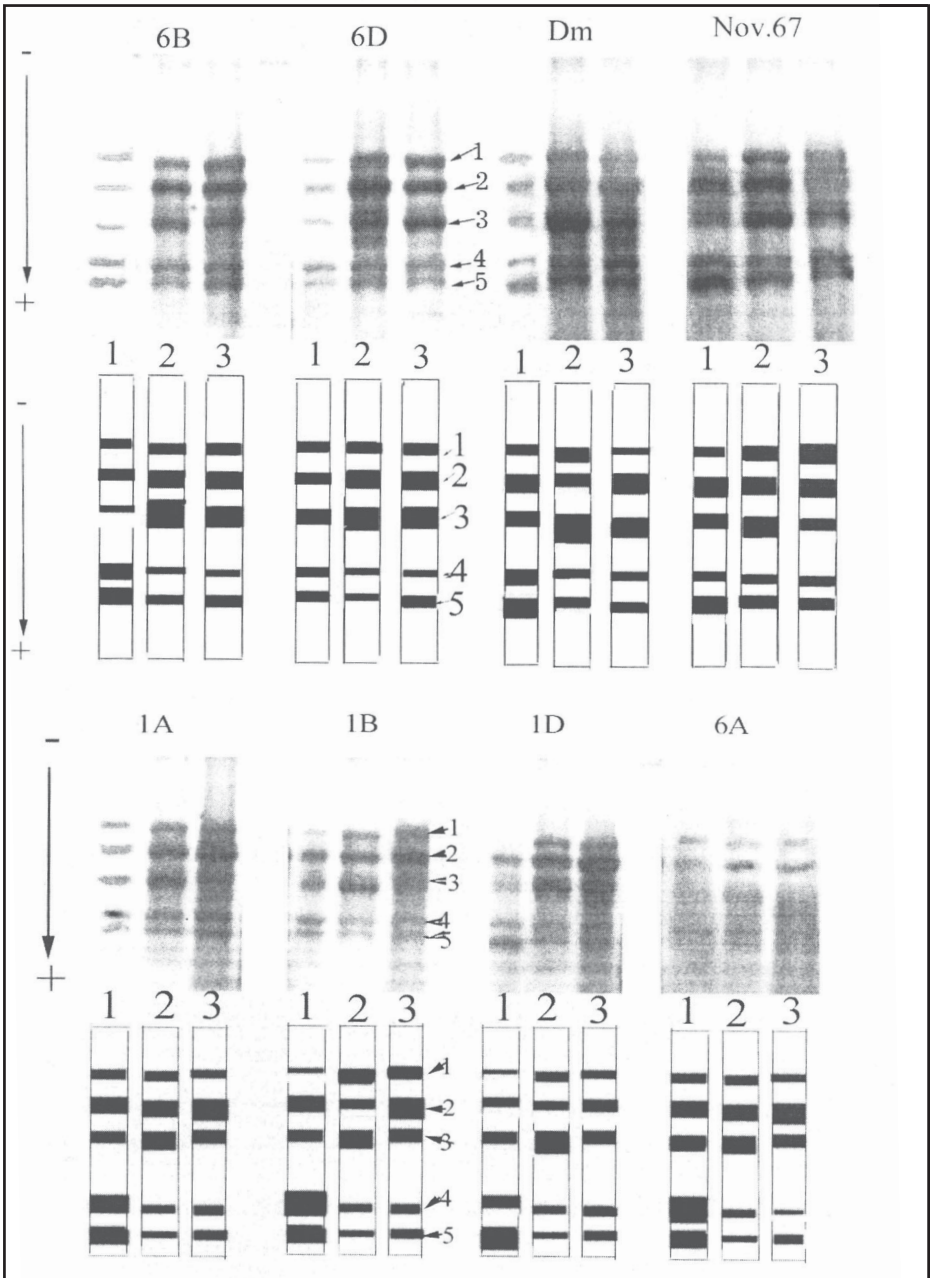

Fig. 9. SDS–PAGE of high-molecular-weight glutenin subunits for the group 1 and 6 homoeologous chromosomes. Lane 1, glutenin subfraction extracted from flour with 0.05 m acetic acid; Lane 2, glutenin subfraction extracted from flour with 4 m urea; and lane 3, glutenin subfraction extracted from flour with 4 m urea and 2-mercaptoetanol.

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Thiol:protein disulfide oxidoreductase (EC 1.8.4.2) in wheat caryopses. A. Substrate specificity of a homogenous preparation.

S.V. Osipova, T.N. Mitrofanova, A.V. Permyakov, and V.A. Trufanov.

According to the actively developed concept of folding, secretory-protein association of wheat storage proteins, their accumulation in protein bodies takes place with the participation of associated endoplasmic reticulum chaperons and enzymes catalyzing formation, isomerization, and dissociation of disulfide bonds (Shewry et al. 1995; Galili et al. 1996; Wang 1998). Indeed, Bulleid and Freedman (1988) demonstrated that the addition of purified protein disulfide isomerase (PDI) to wheat γ-gliadins significantly increases the efficiency of disulfide bond formation in these gliadins. Later, Shimoni et al. (1995) extracted PDI from the endosperm of developing wheat caryopses and proved that it was a glycoprotein with a molecular mass of 60 kD localized in the endoplasmic reticulum and protein bodies. Protein disulfide isomerase expression increases during the height of gluten protein synthesis, between 14 and 28 days-after-flowering, when considered along with data on enzyme localization, also supports its necessity for folding and association of wheat storage proteins. Kobrehel et al. (1992) showed that under physiological conditions the system NADFH/thioredoxin h/thioredoxinreductase catalyzes the in vitro cleavage of disulfide bonds of the same wheat proteins. Formation and dissociation of disulfide bonds in wheat storage proteins were presumed to be affected by the balance between activities of enzymes similar to PDI and thioredoxinreductase (Galili et al. 1996).

In our laboratory, we have extracted the enzyme thiol:proteindisulfide oxidoreductase (EC 1.8.4.2) (TPDO) (Trufanov et al. 1999) from the wheat grain. The molecular mass and subunit composition (unpublished results) of the enzyme indicate that it is not PDI, as described by Shimoni et al. (1995) and not thioredoxinreductase (Kobrehel 1992). The enzyme TPDO catalyzes the dissociation of protein disulfides with reduced glutathione as a cofactor. This enzyme is interesting from the point of view of its possible participation, along with PDI and thioredoxinreductase, in the folding of wheat storage proteins and, ultimately, in the formation of wheat technological characteristics. The present work was targeted at the study of substrate specificity of TPDO from the wheat caryopsis.

Materials and Methods. TPDO was extracted after rough grinding flour of Tulunskaya 12, a Siberian wheat selection, and further purified in accordance with the previously described methods (Trufanov et al. 1999). Activity of TPDO was identified by the speed by which SH-group concentration increases in aliquots of reaction mixture selected with certain time intervals at 36°C. Introduced into the incubation medium as substrates were insulin (Endocrine Preparations Factory, Kaunas), BSA (ICN Pharmaceuticals Inc., Costa Mesa, CA, U.S.A.) in different concentrations, and a fraction of gluten proteins soluble in 0.1 M Tris-HCl buffer containing 1 mM EDTA, 10 % sodium salicylate, the purified enzyme (20 mkM), and the cofactor GSH (1 mM) (Reanal, Hungary). Aliquots were placed in acidulous acetone for protein precipitation. Precipitates were centrifuged at 8,000 X g for 20 min, washed twice with acidulous acetone, and dissolved in 3 ml of 0.1 M Tris-HCl buffer, pH 8.0, containing 1 % Ds-Na. 5, 5'-dithio-bis (2-nitrobenzoic acid) was added to all analytical samples in 0.1 M Tris-HCl buffer, pH 8.0, and after 40 minutes, the optical density was measured at 412 nm. An increase of SH-group concentration/minute (mkmol/min) in the incubation medium was taken for the activity unit. Specific activity was expressed in activity units (E)/mg of enzyme protein, where $E = \Delta SH/t$. Protein concentration was determined according to Lowry et al. (1951). The control did not contain enzyme. The Mikhaelis constant was determined according to Lainuiver-Berk (Dixon and Webb 1982).

To produce gluten protein preparations, finely ground flour was degreased three times in succession by sulfuric and petroleum ether in the proportion 1:3 (w:v) flour:extracting agent and dried. Gluten was first washed with a 0.01 M solution of sodium pyrophosphate Na, pH 7.0, and then with water. Raw gluten was homogenized in 0.1 M acetic acid and lyophilized. Dry gluten powder was dissolved in 0.1 M Tris11-buffer, pH 7.5, containing 5 mM EDTA and 10% Na salicylate and used in the tests.

Results and Discussion. The kinetic curves of the speed of SS-bond cleavage reaction catalyzed by TPDO as a function of substrate concentration (A–insulin, B–BSA) are shown in Fig. 10 (p. 142). With a 1 mM GSH concentration in the incubation medium, the Km calculated according to Lineweaver-Burk for reciprocals of insulin amounted to 2.18 mkM,

which is 5–10 times less than Km values for the analogous enzyme extracted from various animal tissues (Schomburg et al. 1994). The Km for BSA under the same conditions amounted to 570 mkM, which indicates that TPDO has the highest affinity constant to insulin. Data on the dependence of SS-bond dissociation speed on substrate concentration for gluten proteins due to low solubility of these proteins in normal buffers is difficult to obtain. Fig. 11 shows the change of SH-group content over time in an incubation medium containing gluten proteins and 1 mM GSH (Fig. 11, 1), gluten proteins and 1 mM GSH, and TPDO (Fig. 11, 2). If the SH-group content in the incubation medium increases by only 7 % (Fig. 11, 1), the SH-group content increases by almost 40 % with the addition of the TDO enzyme (Fig. 11, 2). Thus, TPDO is able to catalyze dissociation of SS bonds of wheat gluten proteins and apparently participates in the formation of the SH/SS status of wheat reserve proteins.

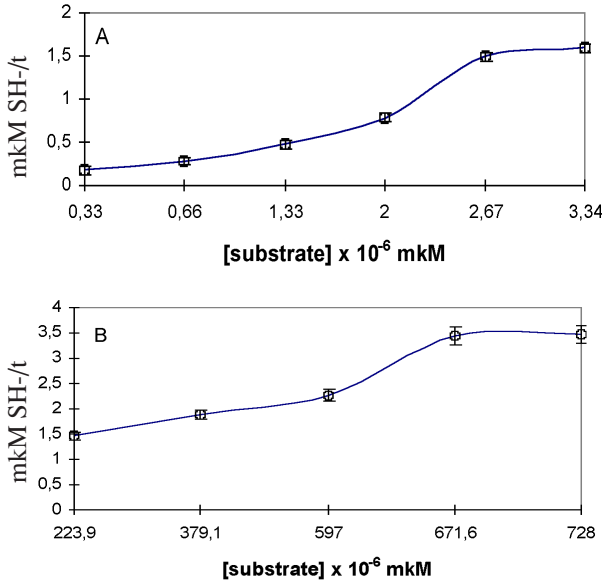

Fig. 10. Activity of thiol:proteindisulfide oxidoreductase as a function of substrate concentration (A, insulin; B, BSA). The average values of three repeated tests are presented.

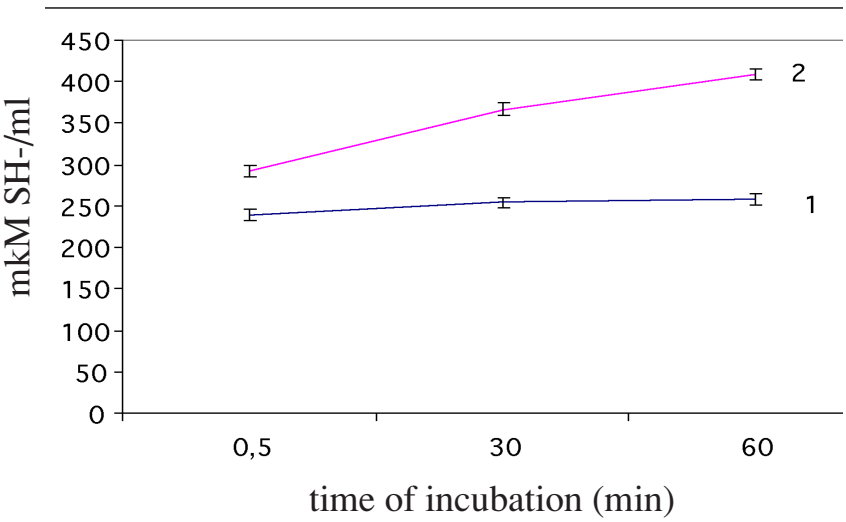

Fig. 11. Change in SH-group concentration (mkM/ml) in soluble proteins of wheat gluten caused by thiol-proteindisulfide oxidoreductase (TPDO). 1. Incubation medium consisting of gluten proteins and GSH cofactor in 0.1 M Tris-HCl buffer and 1 mM EDTA. 2. Incubation medium consisting of gluten proteins, GSH, and TPDO (20 mkM).

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Thiol:proteindisulfide oxidoreductase (EC 1.8.4.2) purified from wheat caryopses. B. Enzymatic characteristics.

S.V. Osipova, A.V. Permyakov, T.N. Mitrofanova, and V.A. Trufanov.

The enzyme thiol:proteindisuldife oxidoreductase (TPDO, EC 1.8.4.2) catalyzing the dissociation of SS-bonds of insulin, bovine serum albumin, and proteins of acetic acid-soluble gluten fraction (Trufanov et al. 1999; Osipova et al., previous article) was extracted from mature wheat grain and purified to electrophoretic homogeneity. The physiological role of this enzyme is assumed to be participation in posttranslational modification of cystine-containing gluten proteins and formation of a gluten macromolecular protein complex. We were interested in studying some enzymatic characteristics of proteindisulfide reductase, the impact of inhibitors on TPDO activity, and comparing the identified characteristics with the properties of identical enzymes of animal origin.

Materials and methods. Proteindisulfide reductase activity was determined as described in the previous article (Osipova et al. 2004). Bovine serum albumin (BSA, ICN Pharmaceuticals Inc., Costa Mesa, CA, U.S.A.) was used as a substrate in the incubation medium at a concentration of 40 mg/ml. Inhibitors included 1 mM phenylmethylsulfonylfluoride (PMSF; Calbiochem, Germany), 0.5 and 1 mM N-ethylmaleimide (NEM; Sigma Chemical, St. Louis, MO, U.S.A.), 0.5 and 1 mM p-chloromercuribenzoate (PCMB; Sigma Chemical, St. Louis, MO, U.S.A.), and 0.5 and 1 mM solutions $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ (Reakhim, Russian Federation). The enzyme was preincubated with inhibitors for 30 minutes at 30°C. Standard methods then were used to determine remaining activity of proteindisulfide reductase. Inhibition percentage was calculated. Dependence of disulfide reductase activity on pH was determined in 0.1 M Tris-HCI buffer + 1 mM EDTA solutions, with pH ranges 5–8 at constant temperature of 36°C. GSH was used as a cofactor at a concentration of 1 mM. Dependence of enzyme activity on temperature was determined in 0.1 M Tris-HCl buffer, pH 7.5, + 1 mM EDTA within a temperature range of 25–46°C.

Results and Discussion. The dependence of disulfide reductase activity on temperature is shown in Fig. 12A. The optimal activity temperature values are 33–38°C; maximum activity was observed at 36°C. Enzymes extracted from animal tissues have approximately the same temperature optimum, 37°C (Varandani et al. 1973; Chandler et al. 1975). Fig. 12B shows that wheat disulfide reductase has an optimum pH between 6.5–7.5, with maximum activity at pH 7.0. At extreme pH values (5.0 and 8.0), about 20 % of maximum activity of disulfide reductase is preserved. The optimum pH values for the wheat enzyme are similar to the optimum for animal disulfide reductase (Ansorge et al. 1973; Tomizawa et al. 1962), only some researchers identify more alkaline optimum pH values, 7.5–8.5 (Chandler et al. 1975; Carmichael et al. 1979). Therefore, with respect to optimum pH and temperature activity, wheat disulfide reductase is similar to the analogous enzymes extracted from animals.

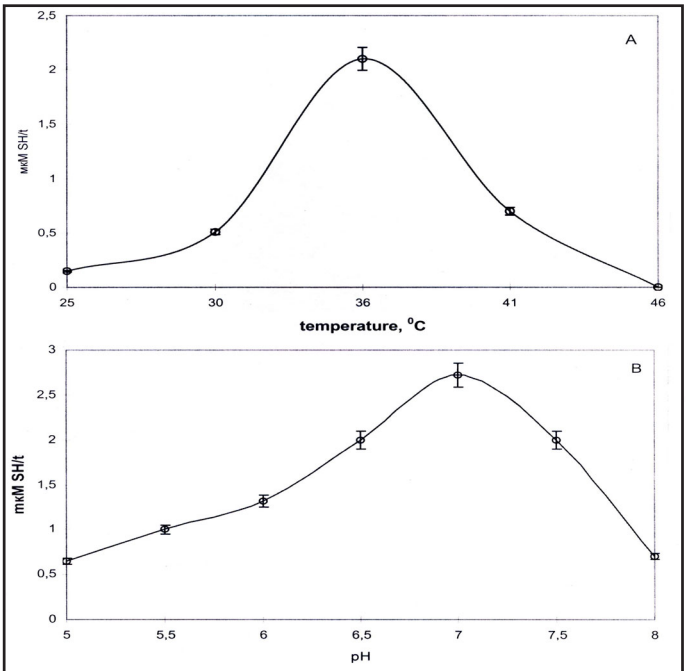

Fig. 12. Thiol:proteindisulfide oxidoreductase activity as a function of temperature (A) and pH (B).

Inhibition tests. Specific reagents for the SH group (Table 9, p. 144) were used for inhibitor analysis. Bivalent ions of copper and zinc linking SH-groups forming mercaptans demonstrate 100% inhibition of disulfide reductase activity. Alkalizing agents, NEM and PCMB also inhibit SS-reductase activity, with NEM being a stronger suppressor, which might be explained by different accessibility of enzyme active center SH-groups for these compounds. We presume from these data that thiol:proteindisulfide oxidoreductase in the wheat caryopsis is a representative of thioredoxin superfamily with a thioredoxin-like domain –Cys–Cly–His–Cys– in the active center (Darby and Greiton 1995). The inhibitor PMSF, a serine protease, also suppresses disulfide reductase activity quite significantly, by 50 %. The serine hydroxyl group also is likely to be important for disulfide reductase activity. Thus, the enzyme

extracted from the wheat caryopsis is similar to animal proteindisulfide reductases by some characteristics (pH and optimum temperature) as well as by inhibition by alkalizing agents and heavy metals ions.

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Table 9. The impact of inhibitors on thiol:proteindisulfide oxidoreductase activity isolated from the wheat caryopsis. NEM is N-ethylmaleimide, PCMB is phenylmethylsolfonylfluoride, and PMSF is p-chloromercuribenzoate.

Inhibitor	Concentration (mM)	Inhibition (%)
$CuSO_4$	0.5	100
	1.0	100
$ZnSO_4$	0.5	100
	1.0	100
NEM	0.5	70
	1.0	100
PCMB	0.5	20
	1.0	53
PMSF	1.0	50

Note: Proteindisulfide reductase was incubated with inhibitors for 30 minutes at 30°C after which the remaining activity was determined by standard methods. The initial enzyme activity in the control was 0.1 mkM SH/min/mg (protein). Results represent the mean of three independent experiments with a standard deviation less than 10 %.

Thiol:proteindisulfide oxidoreductase (EC 1.8.4.2) wheat caryopses. C. Impact on aggregation of wheat storage proteins.

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The ability of wheat storage proteins to aggregate is one of important parameters characterizing the rheological properties of dough and gluten quality (Arakava and Yonezawa 1975; Arakava et al. 1976). The enzyme thiol:proteindisulfide oxidoreductase, isolated from the wheat caryopsis, its substrate specificity, and some enzymatic properties were described in previous articles (pp. 141-144), catalyzes the dissociation of disulfide bonds in gluten proteins. We are interested in the impact of this enzyme on the aggregation properties of the acetic acid-soluble fraction of wheat storage proteins.

Materials and Methods. To determine aggregation, we used the acetic acid-soluble gluten fraction of wheat cultivar Tulunskaya 12 extracted by standard methods. Gluten from preliminarily degreased flour was first washed by 0.1 M solution of sodium pyrophosphate, pH 7.0, and then with water. Raw gluten was dissolved in 0.1 M acetic acid and lyophilized. Aggregation parameters were determined according to the method of Arakawa and Yonezawa (1975). In the control, equal volumes of a gluten-protein solution in 0.1 M acetic acid (final protein concentration 0.01 %) and 0.2 M sodium phosphate buffer, pH 5.6, containing 0.5 M NaCl and 1 mM GSH cofactor were mixed. Thiol:proteindisulfide oxidoreductase (3–5 activity units) was added during the test. The optical density of the solution was measured by spectrophotometry (350 nm) according to the aggregation process time. The following formula was used to calculate aggregation (t10/C, solution turbidity per protein concentration unit for 10 minutes of the processing): $t10 = 2D/C$, where D is the optical density of the solution at $\lambda = 350$ nm and C is the % protein concentration. The results were analyzed by common statistical methods.

Results and Discussion. The results of the test on the impact of thiol:proteindisulfide oxidoreductase on aggregating ability of gluten acetic acid -soluble fraction are shown (Fig. 13). Because wheat disulfidereductase is a glutathione-dependent enzyme, GSH (1 mM) was used in both the control and test substances during the course of the experiment. A test at pH 5.6 recommended by the standard methods (Fig. 13A). The aggregating ability of the protein is reduced after introduction of the enzyme as compared to the control. The aggregation parameter t10/C decreased in the test when compared to control, from 134.1± 4.6 (control) to 114.6 ± 1.2 (test), a 15–22 % decrease. Furthermore, during the 25 minutes of the experiment (until aggregation was terminated), the aggregation parameter in the control was 143.6 ± 5.1 and in the test sample was 119.4 ± 1.2. The test was conducted at pH 7.5, which is optimal for disulfidreductase activity (Fig. 13B). In this case, the aggregation parameter t10/ C decreased by 25–30 % with the addition of the enzyme. The reduction in aggregation parameters is apparently due to the dissociation of S-S bonds in the proteins and the consequent weakening of gluten matrix. Arakawa and Yonezawa (1975) proved that aggregation parameters of strong wheats are considerably higher than those for weak wheats, therefore, a reduction in aggregation parameters after addition of enzyme demonstrates a weakening of the gluten complex and a consequent lowering in gluten technological characteristics. Disulfidreductase apparently may be used to improve quality of extremely strong gluten.

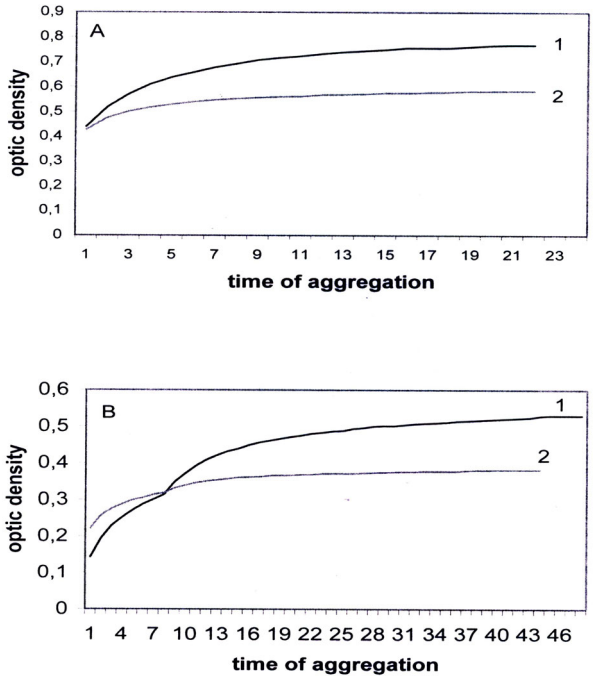

Fig. 13. The impact of thiol:roteindisulfide oxidoreductase on the aggregation of acetic acid-soluble gluten proteins. A, pH 5.6; B, pH 7.5. Line 1 is for the control and line 2 is the test conditions.

We have suggested previously the participation of this enzyme in the regulation of SS/SH proportion in reserve proteins in postsynthetic period (Trufanov and Kichatinova 1989). Thiol:proteindisulfide oxidoreductase is likely to play an important role in storage protein degradation during caryopsis sprouting, as the enzyme reaches a fairly high activity in the mature grain.

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Disulfide reductase activity and gluten quality in common wheat lines with intervarietal substitutions for chromosomes of homoeologous groups 1 and 6.

S.V. Osipova, V.A. Trufanov, and T.A. Pshenichnikova.

The capacity of common wheat, storage proteins for gluten formation is of great importance for breeding. A high storage-protein content is not a guarantee of high gluten quality, the latter being in a substantial dependence on the SS/SH-bond status of these proteins (Bloksma 1975; Kretovich 1991). Wheat grains contain a specific enzyme system belonging to the class of oxidoreductases responsible for thiol-disulfide metabolism in proteins (Trufanov 1994; Trufanov et al. 1999). The study of thiol:protein disulfide oxidoreductase (disulfide reductase, RED, EC 1.8.4.2) and thiol:oxygen oxidoreductase (thioloxidase, EC 1.8.3.2) activities in spring cultivars of wheat with different gluten quality have shown a correlation between the activity and rheological properties of dough. The high genotypic variability in the

specific activity of thioloxidase and disulfide reductase was established in 18 common wheat spring cultivars of different origin, indicating the genetic diversity for this character in wheat (Trufanov et al. 2000). Therefore, we were interested in investigating intervarietal substitution lines with pairs of chromosome of a recipient cultivar substituted for with homologues from a donor cultivar. The results of our study of disulfide reductase (RED) activity and some technological characteristics of grain in substitution lines involving chromosomes of homoeologous groups 1 and 6 of common wheat are presented. These chromosomes are known to carry the genes for storage proteins responsible for gluten complex formation (Wrigley and Shepherd 1973).

Materials and Methods. Substitution lines for chromosomes 1A, 1B, 1D, 6A, 6B, and 6D and the double substitution line 1A and 6D (Maystrenko et al. 1998) were used. The cultivar Diamant (Dm), which has one of the highest grain protein contents but poor technological properties, was the recipient cultivar and the high-quality cultivar Novosibirskaya 67 (N67) was the donor. Disulfide reductase activity was determined according the previously described methods (Kichatinova et al. 1993; Trufanov 1994). Technological parameters were studied as described elsewhere (Trufanov et al. 2000). The data was averaged for two independent replicates of the experiment. The activity of disulfide reductase in each was determined three times in two biological and three analytical replicates. The data on specific activity and technological parameters are shown in percent of the recipient cultivar Dm.

Results and Discussion. According to the modern concept, the physical properties of the gluten-protein complex are determined considerably by the content of intra- and intermolecular S–S bonds in the storage proteins. Their formation, breakage, and isomerization are catalyzed by the specific enzyme system of SS/SH metabolism. One of the key enzymes in this system, RED, catalyzes the reaction of reducing the disulfide bonds and, so far, participates in the formation of SH/SS status of storage proteins. The data of Figs. 14 and 15 show that the DS N67 (1A) Dm (1A) substitution line and the double substitution line DS N67 (1A, 6D) Dm (1A, 6D) have better technological properties, i.e., higher flour strength and extensibility, dough resistance, valorimeter number, and loaf volume and a lower dough dilution. On the whole, the data indicates that in lines with these substitutions, the technological properties have been improved compared to the parental cultivar Dm. At the same time, RED activity was significantly lower in these lines than in the parental. As can be seen from Table 10 (p. 147), RED activity negatively correlates with water-

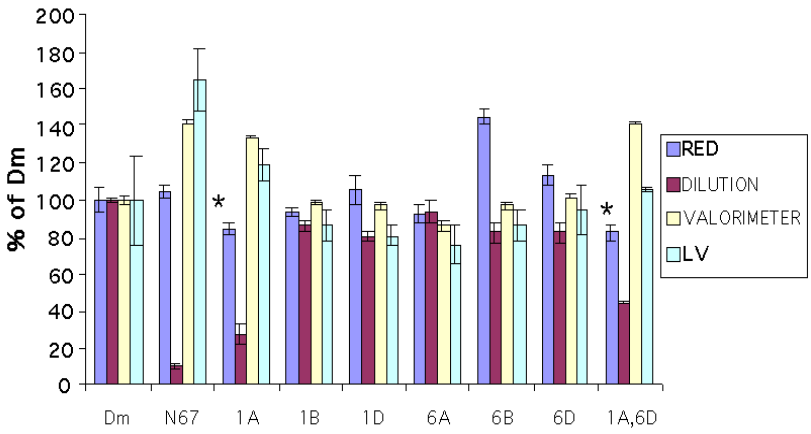

Fig. 14. Specific disulfide reductase (RED) activity, dilution, valorimeter and loaf volume (LV).

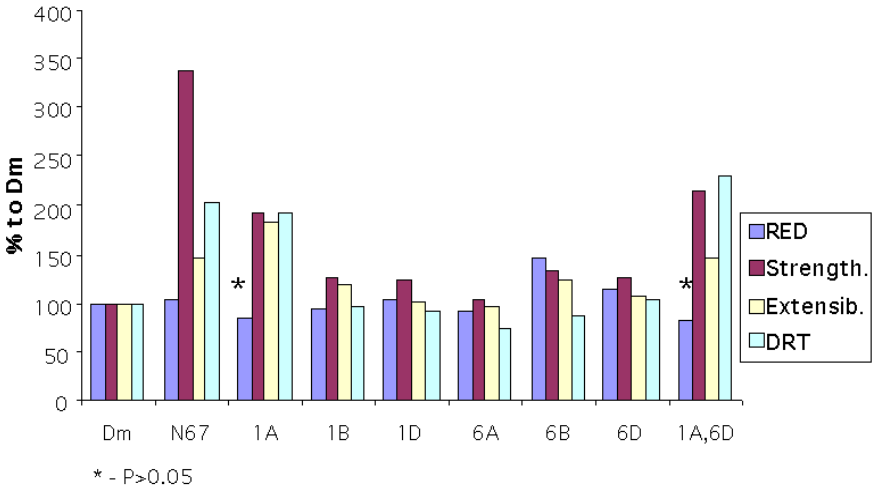

Fig. 15. Specific disulfide reductase (RED) activity, strength, extensibility and dough resistance time (DRT).

Table 10. Significant correlation coefficients for disulfide reductase (RED) activity with respect to quality characteristics. Amounts of RED with an * are significant at $P > 0.05$.

Quality characteristic	RED
Flour water-absorbing capacity	–0.759*
Dough dilution	0.415
Dough resistance	–0. 674*
Valorimeter	–0.444

absorbing capacity, dough resistance, and valorimeter number. A positive correlation with dough dilution is observed, which may be connected with the participation of this enzyme in breaking of S–S bonds in the gluten structural matrix.

Introducing the favorable 'a' allele of *Glu-A1* into a cultivar genotype, e.g., during the production of intervarietal substitution lines, improves the quality (Mansur et al. 1990). Out data shows another result of substitutions involving chromosome 1A, a change in RED activity followed by an improvement of separate technological properties. We have not found any data supporting the chromosome location of genes for RED in cereals. The significant reduction of RED activity in lines DS N67 (1A) Dm (1A) and DS N67 (1A, 6D) Dm (1A, 6D) allows us to propose that these two chromosomes participate either in the direct genetic control of this enzyme or regulate its activity.

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The relationship between specific lipoxygenase activity and technological characteristics of gluten quality in hexaploid wheat.

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Lipoxygenases (linoleat: oxygen oxidoreductase, Lpx, EC 1.13.11.12) are a group of enzymes that catalyze the deoxygenation of polyunsaturated fatty acids containing 1,4-pentadiene system to form a Z,E- conjugated hydroperoxide fatty acid (Grechkin 1998). Lipoxygenases are distributed widely in plants and animals. In animals, lipid hydroperoxides and products of their conversion derived through the Lps pathway are involved in many physiological and pathological processes. Plant lipoxygenase have been implicated in flavor and odor formation. They also may play a role in response to pest attack and wounding (Siedow 1991). Some Lpx isoenzymes also may function as vegetative storage proteins (Tranbarger et al. 1991). The Lpx of wheat is studied mainly in connection with its role in bleaching carotenoid pigment in durum wheat grain (Mc Donald 1979; Pastore et al. 2000). Our interest in Lpx is conditioned by its participation in the formation of superoxide radicals that *in vivo* may oxidize SH-groups of wheat storage proteins with the formation of inter- and intramolecular disulfide bonds stabilizing gluten protein complexes in hexaploid wheat.

The assessment of potential wheat bread-making quality is based on technological procedures including the evaluation of milling properties of grain and physical properties of flour and dough. Among the milling parameters are

1,000-kernel weight and vitreousness. The first trait is the function of grain size, density, and uniformity and is connected with flour extraction during milling. Vitreousness characterizes endosperm consistency and determines the strength of linking and the ratio between starch granules and protein in endosperm. The physical properties of dough measured with alveograph and farinograph can predict the final bread-making quality of wheat. On the basis of alveograph parameters, wheats are classified as having strong or weak gluten. Farinograph characterizes dough resistance to a long machine processing during technological procedures.

Materials and methods. Seeds of 53 RILs and the parental genotypes of the ITMI mapping population were kindly presented by Andreas Börner (Institut für Pflazengenetik und Kulturpflanzenforschung, Gatersleben, Germany). Lipoxygenase activity was analyzed in Tris-soluble flour extracts according to Doderer et al. (1992). The parameter was determined through formation of conjugated super-peroxide compounds of linoleic acid, registered using spectrophotometer at a wavelength of 234 nm. One unit of activity was defined as the change in optical density on 0.001/one minute. Specific activity was expressed by ratio of activity units to 1 mg of protein in 1 ml of incubation media. Protein concentration was determined by the Lowry method (Lowry et al., 1951). The layout of analysis for most traits of grain and flour qualities in recombinant inbred lines corresponds to the Methods of State Variety Testing of Crops accepted in Russia (Anonymous 1988). Thousand-kernel weight was computed per gram dry weight. To investigate vitreousness, 100 grains of each line were cut and classified according to the type of endosperm as vitreous, semivitreous, or floury. The parameter of vitreousness is the percent of vitreous and half of the semivitreous grains.

To determine the physical properties of dough, grain was milled on the COKB mill with flour extraction of 70 % on average. Analysis was after a 10-day flour tempering following the individual conditioning of grain in each line. M. Chopin alveograph was used to study dough strength. Water absorbing capacity, dough resistance, and valorimeter number were measured with farinograph. Correlation between traits was calculated with the help of the Microsoft Excel 2000 computer program.

Results and discussion. The correlation between specific Lpx activity and some quality characteristics are given in tables. From Table 11, we see that Lpx activity is significantly and positively related to 1,000-kernel weight (0.305*), vitreousness (0.318*), and water-absorbing capacity (0.289*).

Table 12 shows that correlating Lpx activity with technological parameters depends on the value levels of Lpx activity. At low values, the specific level Lpx activity shows no significant correlation. At high values, the level of Lpx activity is positively related to 1,000-kernel weight. The correlation of Lpx activity with such parameters as flour strength, resistance to mixing, and valorimeter number were negative at high Lpx values (–0,402*; –0.389*, and –0.381*, respectively).

Table 11. Correlation relationships of specific Lpx activity with some technological parameters of gluten quality.

Trait	r
1,000-kernel weight (g)	0.305*
Vitreousness (%)	0.318*
Dough strength (u.a.)	–0.146
Water-absorbing capacity (%)	0.289*
Resistance to mixing (min)	–0.134
Valorimeter number (u.v.)	–0.178

n=55, mean of specific Lpx activity is 114.2 sp.u. Standard deviation is 39.7 sp.u.
* $P > 0.05$.

Table 12. Correlation relationships of specific Lpx activity with technological parameters in dependence on its value levels.

Trait	Low value level	High value level
1,000-kernel weight (g)	–0.287	0.416*
Vitreousness (%)	0.333	–0.041
Dough strength (u.a.)	0.315	–0.402*
Water-absorbing capacity (%)	0.359	0.003
Resistance to mixing (min)	0.219	–0.389*
Valorimeter number (u.v.)	0.251	–0.381*

Low value level specific Lpx activity, n = 26, mean is 80.3 sp.u., standard deviation is 14.0 sp.u.
High value level specific Lpx activity, n = 29, mean is 144.6 sp.u., standard deviation is 29.0.
* $P > 0.05$.

Besides yield, the quality of the grains harvested is one of the most important characteristics of the wheat crop. The composition of endosperm storage proteins, gliadins and glutenins, to a great extent determine the final quality parameters of wheat flour (Payne et al., 1979). However, most gluten physical properties correlate with the content of disulfide bonds in the

storage proteins, which are responsible for stabilizing space structure of the gluten complex (Lasztity 1980; Trufanov 1994). Wheat seed contains a specific system of enzymes, referred to as an oxidoreductase class, and directly or indirectly regulate thiol-disulfide metabolism in proteins (Shiiba et al. 1991). Some of them catalyze the formation of SS bonds, and the level of their activity correlates with certain technological properties of grain (Trufanov et al. 1999). Lipoxygenase is one of such enzymes that can indirectly, through formation of peroxides and hydroperoxides of unsaturated fatty acids, oxidize SH groups of proteins with the formation of SS bonds. According to our data, we concluded that specific Lpx activity is related to the quality of wheat gluten. This relationship depends on the value levels of Lpx activity. Detailed technological and biochemical analysis of different parameters involved in wheat flour quality seem to be a very productive areas of research and will allow us to more successfully separate the quality into different genetic components and to use molecular techniques to map them for breeding purposes.

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Characterization of transgenic wheat plants overexpressing chloroplastic iron-superoxide dismutase.

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Earlier studies on genetic modification of plant antioxidant (AO) defense systems led to largely controversial results regarding the possibility of strengthening plant resistance to most frequent stress factors via transferring SOD gene(s) to plants by *Agrobacteria*-mediated transformation methods (Tepperman et al. 1990; Bowler et al. 1991; Pitcher et al. 1991; Van Camp et al. 1996; McKersey et al. 1996; McKersey et al. 1999). With this in mind, it is of considerable scientific interest both to use alternative methods of plant genetic transformation while introducing AO-defense genes and to enlarge the list of higher plant species used for introduction of relative transgenes. The latter looks particularly important because the systematic and evolutionary position of the species undoubtedly affects transgene manifestation and physiological-biochemical consequences of its introduction in the genome. The present work looks at the acquisition and compilation of physiological-biochemical characteristics of wheat transgenic plants with *Arabidopsis* FeSOD gene introduced by biolistic transformation.

Materials and methods. Plants of the wheat cultivars Tulunskaya-12 and Skala were used in this research. The procedure for wheat plant transformation by biolistic method is described below. Isolated immature wheat embryos (24–28 days-after-pollination) were placed in Petri dishes 1–2 hours prior to bombardment on an agar medium of the following composition: one-half Murashige and Scoog salts (Murashige and Scoog 1962), 3 % sucrose, 0.125 M sorbite, and 0.125 M mannitol. The extraction of pEXSOD10 plasmid DNA (construct kindly provided by Dr. Marc van Montagu, Gent, Belgium) from JM 103 *E. coli* cells was performed according to the method (Birnboim and Doly 1979). Tungsten powder with an average particle diameter of 1.1–1.2 mm (BioRad, Emeryville, CA, USA) was used as microbullets. The DNA–tungsten suspension was prepared and placed on microbullets immediately prior to bombardment according to the method of Sanford et al. (1993). Bombardment was performed from the pneumatic gene gun of original design (Siberian Institute of Plant Physiology and Biochemistry of the Russian Academy of Sciences, Irkutsk). Two to three days after control embryos (not subjected to shooting but kept in the same conditions) and transformed embryos were placed in Magenta vessels for sprouting in the light in room conditions on the nutrient medium containing Murashige and Scoog mineral salts with the addition of 3 % sucrose. Immature embryos formed seedlings by direct organogenesis on average in 15 days. In order to obtain fertile plants (R_0-plants), 15–20-day-old seedlings were planted in a phytotron in claydite, where nutrient solution was introduced.

DNA was extracted from transformed and control wheat plants according to the method of Konieczny and Ausubel (1993) with small modifications. DNA was analyzed by PCR for the presence of an insertion corresponding to *Arabidopsis* FeSOD gene using a pair of specific primers synthesized on the basis of cDNA sequence corresponding to the FeSOD gene transcript from *A. thaliana* (Van Camp et al. 1996)

5' – GATCTAAACTACGTCCTCAAGCCACC – 3' (5' primer) and
5' – GGATCCCACACTCAGAAAAGAGCATG – 3' (3' primer).

In the course of DNA analysis by PCR, we also was used an alternative approach. A pair of primers (so-called combined primers) specific to the fragment embracing the 3' region of the *Arabidopsis* FeSOD gene and the 5' region of neomycin phosphotransferase II gene (NPT II) within the insertion

5' – GGTGGCTTGAGGACGTAGTTTAGATC – 3' (5' primer) and
5' – GATGCGCTGCGAATCGGG – 3' (3' primer).

PCR was in 25 ml tubes containing 50 ng of wheat DNA, 2.5 units of Taq polymerase, 0.2 mM dNTPs, 2mM $MgCl_2$, 0.4 pmol/ml of each primer, and 2.5 ml 10 X buffer for Taq polymerase (buffer composition: 670 mM Tris–HCl, pH 8.8, 166 mM $(NH_4)_2SO_4$, 0.1 % Tween 20). After DNA denaturation at 94°C within 5 min, 36 amplification cycles were conducted (denaturation at 94°C, 0.7 min, annealing at 55°C, 1.0 min, extension at 72°C, 1.5 min). The reaction was completed by heating at 72°C within 10 minutes. Amplification products were separated by electrophoresis in 1 % agarose gels, dyed with ethidium bromide, and photographed in UV-light.

SOD activity was determined according to the method of Paoletti et al. (1986). Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. The plants were tested for resistance to increased concentrations of superoxide radicals via exposition of leaf discs of control and transformed plants in 0.1 % Tween 20 solution with addition of different paraquat concentrations (0.1–1.0 mM).

Results and discussion. In pEXSOD10, the genetic control is by the 35S promoter of cauliflower mosaics virus. Unlike agrobacterial transformation in earlier investigations of plant species with Sod genes (Tepperman et al. 1990; Bowler et al. 1991; Pitcher et al. 1991; Van Camp et al.1996; McKersey et al. 1996; McKersey et al. 1999), our present research attempted to transform wheat by biolistic method.

Immature isolated embryos of the spring wheats Tulunskaya-12 and Skala were used for genetic transformation. After biolistic transformation, immature wheat embryos of test and control groups formed plants (R_0). Electrophoretic analysis of PCR products obtained by amplification, using DNA of transformed and control wheat plants of Tulunskaya-12 as a template, demonstrated the presence of a relevant size (~ 820 bp) DNA-insertion in plant regenerants, unlike those of control group (Fig. 16, p. 151). In the course of our PCR-analysis of DNA of transformed and control plants, we used two pairs of primers, which allowed us to analyze different regions of the insertion carrying FeSOD *A. thaliana* gene. Although the first pair of oligonucleoitides was selected for the analysis of the proper coding part of *Arabidopsis* FeSOD gene (Fig. 16), the second pair (so-called combined primers) allowed us to identify the presence of a DNA fragment embracing the 5' region of the *Arabidopsis* FeSOD gene and the 5' region of NPT II gene inside the insertion. The results of the PCR analysis of DNA from transformed and control plants of Skala wheat using combined primers confirmed the presence of a corresponding polynucleotide insertion of 2.5 kb in the transformed plants (Fig. 17, p. 151).

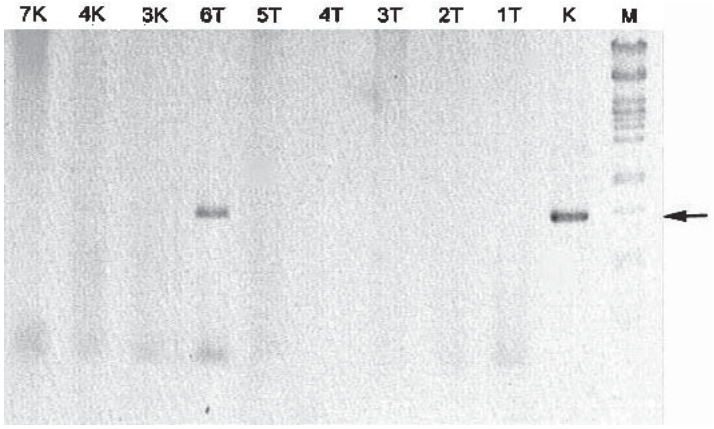

Fig. 16. Results of PCR of DNA of transformed wheat plants of Tulunskaya 12 wheat for the identification of the transgene using primers specific for the *A. thaliana* FeSOD gene. Lanes 3K, 4K, and 7K are control plants; lanes 1T, 2T, 3T, 4T, 5T, and 6T are transformed plants; lane K is pEXSOD10 (positive control); and lane M is the molecular marker/Pst1. The arrow indicates the position of the PCR product corresponding to the *A. thaliana* FeSOD gene by size.

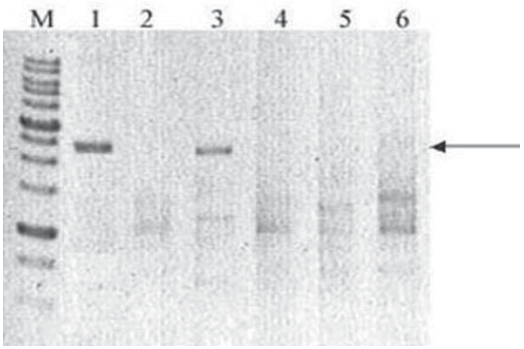

Fig. 17. Results of PCR analysis of DNA of transformed wheat plants of Skala wheat using combined primers. Lane M is the molecular size marker (100-bp ladder); lane 1, pEXSOD10 (positive control); lanes 2–4, transformed plants; and lanes 5–6, control plants. The arrow indicates the 2.5-kb fragment.

In our view, using combined primers made possible a much more reliable identification of transgenic insertion. Total SOD activity in the transformed wheat plants of Tulunskaya 12 was found to exceed the same parameter in the plants of control group by 30 % (Table 13). Nevertheless, we found no differences in the total SOD activity in the seedlings of the R_0 generation of test and control groups of Skala wheat (data not presented). Transformed plants of both Skala (Fig. 18) and Tulunskaya 12 (Fig. 19) wheat possessed much higher (as compared to control) antioxidant activity manifested for all paraquat concentrations used (0.1–1.0 mM).

Table 13. SOD activity in wheat seedlings (cultivar Tulunskaya 12) subjected to biolistic transformation by pEXSOD10 construct with the *A. thaliana* FeSOD gene. The data were acquired in one of two series of independent tests with similar results. SOD activity in the groups of control and transformed plants was determined in a sample obtained from sprouts of 12–15 wheat seedlings grown in sterile conditions. The number of seedlings used for the enzyme extract is given in brackets. The differences are reliable at $P < 0.05$.

Test variant	Specific SOD activity (μ/mg of protein)	% (of control)
Control plants (12)	79 ± 3.6	100
Transformed plants (15)	104 ± 3.8*	131

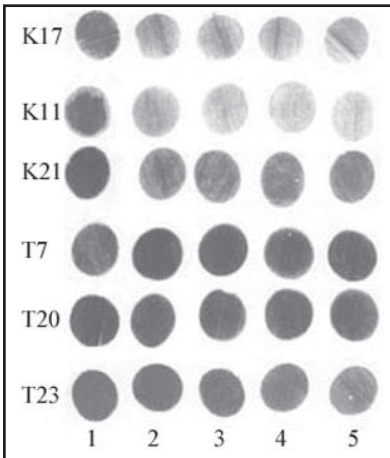

Fig. 18. Determination of paraquat sensitivity in leaves of transformed and control plants of Skala wheat. K17, K11, and K21 are control plants; T7, T20, and T23 are transformed plants. Paraquat concentrations are 1, 0 mM; 2, 0.125 mM; 3, 0.250 mM; 4, 0.5 mM; and 5, 1 mM.

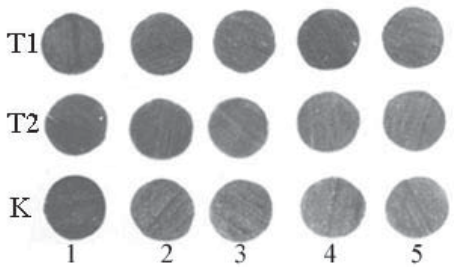

Fig. 19. Determination of paraquat sensitivity in the leaves of transformed and control plants of Tulunskaya 12 wheat. K, control plant; T1 and T2, transformed plants. Paraquat concentrations are the same as those in Fig. 18.

Biolistic transformation of immature wheat embryos with the pEXSOD10 construct provided the transfer of the *Arabidopsis* FeSOD gene to wheat plants. The result was an increase of total SOD activity in seedlings and an enhanced resistance to paraquat in mature plants. During ontogenesis, transformed wheat plants of the R_0 generation demonstrated normal growth and development and produced seeds.

Acknowledgments. The authors are indebted to Prof. Marc van Montagu and his colleagues for kindly providing pEXSOD10 construction used in this study.

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Analysis of antimicrobial peptides from seeds of Triticum kiharae Dorof. et Migusch.

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All living organisms have evolved different defense mechanisms against pathogen attack. The synthesis of antimicrobial peptides belongs to the most widespread and ancient defense strategies. Eight families of antimicrobial peptides, ranging in size from 2 to 9 kD, have been identified in plants (Garcia-Olmedo et al. 1998). These peptides are thionins, defensins, lipid-transfer proteins, hevein, and the knottin-like peptides, MBP1, IbAMP, and snakins. All have compact

structures stabilized by 2–8 disulfide bonds and represent permanent and inducible defense barriers. Considerable progress has been made recently in the identification of antimicrobial peptides in different plant species. However, wild relatives of wheat and related species are poorly studied. In this work, we analyzed the peptide composition of seeds of *T. kiharae*, which is highly resistant to most fungal pathogens.

Materials and methods. The peptide fraction was extracted from *T. kiharae* flour with acid (1 % trifluoroacetic acid, 1 M HCl, and 5 % HCOOH) for 1 h at room temperature. The extract was subjected to chromatography on Heparin Sepharose. Proteins and peptides were eluted with a stepwise NaCl gradient. The fraction was eluted with 100 mM NaCl and further separated on a Superdex peptide column followed by RP-HPLC chromatography of the peptide fraction. The chromatographic fractions were tested for the antifungal activity against *H. sativum* and characterized by mass spectrometry (MS) and sequencing.

Results and discussion. The analysis of the peptide fraction by MS and N-terminal sequencing showed that seeds of *T. kiharae* contain several families of antimicrobial peptides with molecular masses from 3 to 6 kD. We isolated two thionins whose N-terminal sequences differed from the sequences determined earlier for the hexaploid seeds of the *Aegilops–Triticum* group, four new glycine-rich peptides, six new defensins, two new hevein-like peptides, two lipid-transfer proteins, and several peptides whose N-terminal sequences revealed no homology to the known proteins. Tests for the antifungal activity of the glycine-rich peptides found earlier in the roots of only shepherd's purse (*Capsella bursa-pastoris*) (Park et al. 2000) showed that they caused morphological changes in the filamentous fungus *H. sativum*. Defensins isolated from *T. kiharae* seeds had no effect on the growth and morphology of this fungus.

Our data indicate that *T. kiharae* is a valuable source of antimicrobial peptides, whose biological activities will be investigated in more detail.

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A genealogical analysis of the genetic diversity in Russian spring durum cultivars.

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We studied the genetic diversity of the Russian spring durum cultivars with the help of a genealogical analysis. The 79 spring durum cultivars released in the Russian Federation between 1929 and 2003 are given in Table 1 (pp. 154-155). Three cultivars with incomplete pedigrees (Zarnitsa Altaya, Omskaya Yantarnaya, and Orenburgskaya 21) were excluded from the analysis. The dynamics of change in diversity over space and time were estimated on genetic profiles. Pedigree analysis, calculation of coefficients of parentage matrix, and genetic profiles were made with the aid of the Information and Analytical System of Genetic Resources of Wheat GRIS 3.5 (Martynov and Dobrotvorskaya 2000).

We have constructed the genetic profiles for 76 cultivars to analyze change in diversity. The pool of original ancestors of Russian spring durum wheats for the period in totals 92 landraces and old cultivars, including 41 from the Russian Federation. More than half were derived from the original ancestors from European countries (57 %), including 45 % from the Russian Federation and the Ukraine. The number of ancestors from other continents is less: Asia, 24 %; United States, 12 %; and Africa, 7 %. The number of lines with unknown pedigrees is rather small (11.9 %). The original ancestors strongly differ in frequency and, thus, on their importance in the gene pool of Russian spring durum wheats. Landraces, usually indicated with an LV-, such as Beloturka, Sivouska, and Kubanka; original ancestors of Kharkovskaya 46 (LV-Volga region, LV-*T. turgidum*, and LV-*T. dicoccum* from the Kharkov province), and LV-

Zolochevskij district (via Narodnaya); original ancestors of Melanopus 1932; Zabajkalskaya polba; Yaroslav emmer; and the *T. aestivum* cultivar Poltavka are found in the pedigrees more than half of cultivars created in various institutes. About half of original ancestors (46 %) are present in the pedigrees of only 1–2 cultivars (frequency of presence < 3 %). The average contribution of an original ancestor varies from 0.0932 for the LV-Volga region to 0.0001 for Carosella.

The tendency of change in diversity over time is revealed by the analysis of a series of matrixes '*n x m*', where *n* is the number of released cultivars and *m* is the number of original ancestors. For this analysis, we constructed genetic profiles for all cultivars that were released in the Russian Federation between 1929 and 2003 and generated 75 matrixes. We now can see changes in the structure of the original ancestors in sets of cultivars released in any one year (see Fig. 1, p. 156). The pool of original ancestors of the Russian spring durum cultivars for all periods totaled 92 landraces and old cultivars. The process of accumulating landraces in pedigrees (top curve) was accompanied by their loss (bottom curve). The result was that the actual number of landraces in pedigrees over the years increased from four in 1930 to 71–73 in 2000.

The dynamics of the average genetic profile for released cultivars, i.e., an average of the number of original ancestors/pedigree, showed that until 1970, pedigrees contained only 1–2 landraces or old cultivars. Since 1970, pedigrees have become complicated, and the average number of original ancestors/cultivar increased. The

Table 1. Spring durum cultivars released in the Russian Federation between 1929 and 2003 (Table is continued on p. 155).

Cultivar Name	Years of cultivation	Breeding institute [1]	Region [2]
Hordeiforme 10	1929–66	Siberian ARI	5, 6, 9, 10
Hordeiforme 189	1929–66	Krasnyj Kut BS	7, 9
Melanopus 69	1929–71	Krasnyj Kut BS	5, 6, 7, 8, 9
Hordeiforme 432	1929–76	South-East ARI	8
Hordeiforme 27	1933–58	Krasnodar ARI	6
Chaltyrka	1938–39	Don Breeding Centre	6
Samarka	1938–44	—	6
Garnovka Kurskaya	1939–48	—	5
Turka	1939–54	—	5
Donskaya Garnovka 672	1942–62	Don Breeding Centre	6
Hordeiforme 1404	1946–62	Volga ARI	7
Palestinka 6	1947–56	—	6
Hordeiforme 496	1947–61	Gorkij BS	4, 7
Arnautka Nemerchanskaya	1947–71	Nemerchanskaya BS	4
Narodnaya	1947–78	Ukrainian RICP	5, 7, 9
Hordeiforme 4	1948–61	Centr. Chernoz. ARI	7
Leucurum 33	1948–68	Samara ARI	6, 7
Melanopus 1932	1950–70	Krasnyj Kut BS	8
Chakinskaya 226	1951–71	Tambov BS	5
Kubanka 3	1953–71	Krasnodar ARI	6
Krasnokutka	1954–59	Krasnyj Kut BS	8
Hordeiforme 5695	1954–73	South-East ARI	8
Rostovskaya 25	1955–62	Don Breeding Centre	6
Krasnodarskaya 362	1955–89	Krasnodar ARI	6
Chelyabinskaya	1956–65	Chelyabinsk ARI	9
Melanopus 26	1956–86	Krasnyj Kut BS	8, 9
Kharkovskaya 46	From 1957	Ukrainian RICP	5–11 (now 7, 9)
Chernokoloska 1	1960–64	Siberian ARI	10
Raketa	1962–73	Krasnoyarsk ARI	11
Bezenchukskaya 102	1963–67	Samara ARI	7
Bezenchukskaya 105	1965–70	Samara ARI	7
Melanopus 7	1972–85	Krasnodar ARI	6
Zernigradskaya 39	1973–83	Don Breeding Centre	8
Saratovskaya 40	1974–93	South-East ARI	8
Krasnokutka 6	From 1974	Krasnyj Kut BS	8
Leucurum 43	1975–78	South-East ARI	8
Saratovskaya 41	1975–82	South-East ARI	7
Almaz	1979–96	Siberian ARI	10
Altajka	1980–2000	Altaj ARI	10,11
Bezenchukskaya 139	From 1980	Samara ARI	5,7,8,9
Bashkirskaya 17	1982–84	Bashkirian ARI	9
Orenburgskaya 2	From 1982	Orenburg ARI	6, 9
Kharkovskaya 3	1983–99	Ukrainian RICP	8
Luganskaya 7	From 1985	Lugansk BS	5
Svetlana	From 1987	Centr. Chernoz. ARI	5, 8, 9
Orenburgskaya 10	From 1989	Orenburg ARI	5, 7, 9, 10
Saratovskaya 57	From 1989	South-East ARI	8
Persianovskaya 115	1990–2000	Don AU	6
Kharkovskaya 17	1990–2000	Ukrainian RICP	6
Hordeiforme 53	1990–present	Altaj ARI	10
Altajskaya Niva	1991–present	Altaj ARI	10

Table 1 (continued). Spring durum cultivars released in the Russian Federation between 1929 and 2003.

Cultivar Name	Years of cultivation	Breeding institute [1]	Region [2]
Kollektivnaya 2	1991–present	Ukrainian RICP	9
Omskij Rubin	1991–present	Siberian ARI	7, 10
Saratovskaya 59	1992–present	South-East ARI	8
Kharkovskaya 21	1993–2000	Ukrainian RICP	5
Bezenchukskaya 182	1993–present	Samara ARI	7, 8, 9, 12
Voronezhskaya 7	1993–present	Centr. Chernoz. ARI	5, 7
Krasnokutka 10	1993–present	Krasnyj Kut BS	5, 7, 8, 9
Novodonskaya	1993–present	North Donets BS	6
Saratovskaya Zolotistaya	1993–present	South-East ARI	8, 9, 10
SID 88	1993–present	Kustanaj ARI	9
Bezenchukskij Yantar	1995–present	Samara ARI	9
Damsinskaya 90	1995–present	Kazakhstan GRI	9
Lyudmila	1995–present	South-East ARI	8
Kharkovskaya 23	1995–present	Ukrainian RICP	5, 6, 8, 9
Zarnitsa Altaya	1996–present	Altaj ARI	10
Angel	1997–present	Siberian ARI	10, 11
Valentina	1998–present	South-East ARI	8
Step 3	1998–present	Centr. Chernoz. ARI	5, 7, 9, 11
Omskaya Yantarnaya	1999–present	Siberian ARI	10
Nik	2000–present	South-East ARI	8
Altajskij Yantar	2001–present	Altaj ARI	10
Bezenchukskaya 200	2001–present	Samara ARI	7
Voronezhskaya 9	2001–present	Centr. Chernoz. ARI	10
Elizavetinskaya	2002–present	South-East ARI	8
Volnodonskaya	2003–present	North Donets BS	6, 8
Zolotaya Volna	2003–present	South-East ARI	8
Omskij Korund	2003–present	Siberian ARI	10
Orenburgskaya 21	2003–present	Orenburg ARI	9

[1] Abbreviations used: ARI = Agricultural Research Institute, AU = Agricultural University, BS = Breeding Station, GRI = Grain Research Institute, RICP = Research Institute of Plant Production; and — = origin unknown.

[2] Regions include 1 = Northern, 2 = Northwestern, 3 = Central, 4 = Volga–Vyatka, 5 = Central Chernozem, 6 = North Caucasus, 7 = Middle Volga, 8 = Lower Volga, 9 = Ural, 10 = Western Siberia, 11 = Eastern Siberia, and 12 = Far East.

genetic profiles of modern Russian spring durum cultivars in the 2000s include, on average, 9–10 original ancestors. The Shannon diversity index increased over the years from H = 1.20 in the 1930s to 2.90 in 2000s. Thus, during last 75 years, an increase in genetic diversity took place. A detailed analysis during this period, however, indicated that about 20 landraces were lost (Table 2, p. 156). The lost part makes up 20 % of the pool of original Russian wheat ancestor cultivars. We assume that some of them carry a complex of genes for adaptability to specific climatic conditions of the Russian Federation.

Thus, in addition to the increase in genetic diversity, there is genetic erosion of local material in gene pool of the Russian spring durum wheats.

Comparison of the diversity in spring durum cultivars developed by various institutes. For this analysis, we looked at the cultivars of six of the most productive breeding institutes, Agricultural Research Institute for South-East (Saratov), Krasnyj Kut Breeding Station (Saratov province), Samara Agricultural Research Institute, Siberian Agricultural Research Institute (Omsk), Agricultural Research Institute for Central Chernozem Region (Voronezh province), and the Ukrainian Research Institute of Plant Production (Kharkov). The number of cultivars from other institutes was not sufficient for statistical analysis.

To the analysis of the specificity of the distribution of original ancestors in breeding programs of various institutes, we applied a two-way ANOVA of the contributions of dominant ancestors in unorganized replications (Table 3, p. 156). Twelve landraces with the maximum frequency of presence (not less than 50 % in cultivars developed in one or more institutes) were used. The factors investigated were institute (factor *A*) with gradation number a = 6 and dominant original ancestors (factor *B*) with gradation number b = 12. The influence of both factors and their interaction was significant. A significant interaction (*A x B*) shows a distinction in the contribution of dominant ancestors in cultivars developed by various institutes and, thus, the specificity of original ancestors in the different breeding programs (Table 4, p. 157).

The most important ancestors at the Ukrainian Research Institute of Crop Production are original ancestors of the cultivar Kharkovskaya 46 (landraces LV-Volga region, LV-*T. turgidum*, and LV-*T. dicoccum* from Kharkov province),

Table 2. The lost part of the landrace pool of the Russian spring durum cultivars. The year indicates that after which the landrace disappeared from cultivar pedigrees.

Landrace	Year
LV-Rostov region (via Chaltyrka)	1939
LV-Rostov region (via Samarka)	1944
LV-Kursk region (via Turka)	1954
Persian Red	1956
LV-Krasnyj Kut district (via Krasnokutka)	1959
LV-Saratov region (via Krasnokutka)	1959
Deves (LV-GRC)	1961
LV-Simbilej district (via Hordeiforme 496)	1961
LV-Ashhabad region (via Erythrospermum 841), *T. aestivum*	1962
Kakhetinskaya vetvistaya	1964
Accession from Siberian ARI	1965
LV-Samara region (via Hordeiforme 675)	1967
LV-Rostov region (via Kubanka 3)	1971
LV-Urazovskij district of Voronezh region (via Chakinskaya 226)	1971

Table 3. The two-way ANOVA of the contributions of dominant original ancestors of spring durum cultivars on institutes (original data are transformed through arcsin√x). Items with an * are significant at $P < 0.001$.

Source	SS	DF	MS	F
General	71,402.5	539		
Institutes (Factor *A*)	2,471.1	5	494.23	5.37*
Ancestors (Factor *B*)	7,699.4	11	699.95	7.61*
Interaction (*A x B*)	18,171.6	55	330.39	3.59*
Error	43,060.4	468	92.01	

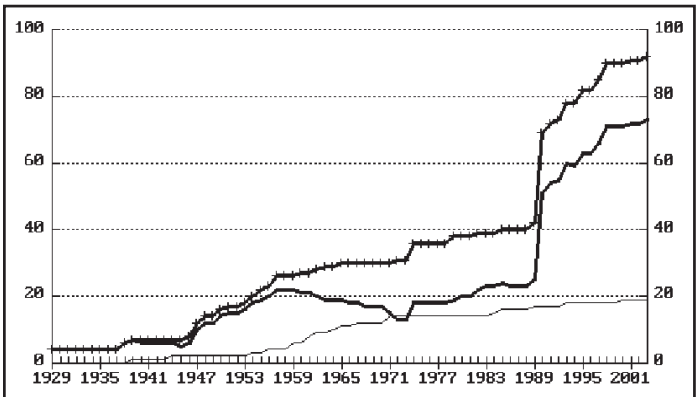

Fig. 1. Dynamics of change in the number of landraces in the pedigrees of spring durum cultivars released in the Russian Federation between 1929–2003. The top curve displays dynamics of landrace accumulation, the bottom line the number of lost landraces, and the middle line the actual landrace number.

and LV-Zolochevskij district of the Kharkov province via cultivar Narodnaya. Among the cultivars developed at Krasnyj Kut Breeding Station, dominant roles belong to Sivouska, the LV-Novouzensk district of the Saratov province, landraces Melanopus and Hordeiforme from the Krasnyj Kut district via cultivar Melanopus 1932, and Kubanka. Sivouska and Beloturka prevail in cultivars of the Samara Agricultural Research Institute. A feature of the Samara breeding program was wide use of Poltavka and landrace *T. aestivum L.* from Saratov province, which enters into pedigrees all released cultivars. In cultivars from the Agricultural Research Institute for South-East Regions, the highest frequency and greatest average contribution is from landraces from Saratov province. Beloturka has high quality grain. The landrace of next importance is Sivouska. In cultivars from the Ukrainian Research Institute of Crop Production and the Agricultural Research Institute for Central Chernozem Region, the ancestors of the greatest importance are those of cultivar Kharkovskaya 46. Also important ancestors in cultivars from this institute are Kubanka and Zabajkalskaya polba (*T. dicoccum)*. In cultivars of the Siberian Agricultural Research Institute, no dominant ancestors are obvious based on the frequency of presence or the average contribution, although the general number exceeds 37 for the number of original ancestors in released cultivars of other institutes (the Krasnyj Kut Breeding Station, 13 landraces; the Agricultural Research Institute for South-East Regions, 20 landraces; the Agricultural Research Institute for Central Chernozem Region, 21 landraces; and the Samara Agricultural Research Institute, 29 landraces).

The similarity of cultivars developed within the framework of the various breeding programs have an effect on the coefficient of parentage between all possible pair combinations. The ANOVA of coefficient of parentage (Table 5, p. 157) shows the high significance of distinction between institutes. The genetic similarity of cultivars developed at the various breeding institutes differ significantly (Table 6, p. 157).

The greatest similarity is that of half-sibs of cultivars developed at the Ukrainian Research Institute of Plant Production. The high value for the average coefficient of parentage for the set of Kharkov cultivars shows that during several decades, continuity in the breeding programs was

Table 4. The contributions of dominant ancestors of spring durum cultivars. Values (inside a line) followed by a different letter are significantly different at $P = 0.05$ probability level by the Duncan range test. The comparisons are made after transformation of the original data through arcsin√x. Institutes include URICP, Ukrainian Research Institute of Plant Production (Kharkov); KKBS, Krasnyj Kut Breeding Station (Saratov province); SARI, Siberian Agricultural Research Institute (Omsk); BARI, Samara Agricultural Research Institute (Bezenchuk); ARISER, Agricultural Research Institute for South-East Regions (Saratov); and ARICC, Agricultural Research Institute for Central Chernozem Region (Voronezh province).

	Institute					
Landrace	URICP	KKBS	SARI	BARI	ARISE	ARICC
Beloturka (LV-Saratov region)	0.00 a	0.01 a	0.00 a	0.11 bc	0.21 c	0.01 a
Kubanka	0.02 a	0.11 ab	0.01 a	0.00 a	0.05 ab	0.11 b
Sivouska (LV-Novouzensk district)	0.00 a	0.16 cd	0.00 a	0.16 d	0.08 bc	0.00 a
LV-Volga region	0.24 c	0.05 a	0.06 a	0.11 ab	0.06 a	0.16 c
LV-Kharkov region *T. turgidum*	0.17 c	0.03 a	0.03 ab	0.06 ab	0.06 ab	0.08 bc
LV-Kharkov region *T. dicoccum*	0.17 c	0.03 a	0.03 ab	0.06 ab	0.06 ab	0.08 bc
LV-Zolochevskij district, Kharkov region (via Narodnaya)	0.18 b	0.00 a	0.00 a	0.00 a	0.04 a	0.03 a
LV-hordeiforme(via Melanopus1932)	0.00 a	0.12 b	0.00 a	0.00 a	0.02 ab	0.00 a
LV-melanopus (via Melanopus1932)	0.00 a	0.12 b	0.00 a	0.00 a	0.02 ab	0.00 a
Zabajkalskaya polba *T. dicoccum*	0.01 a	0.00 a	0.01 a	0.00 a	0.02 a	0.05 a
Poltavka (LV-Saratov) *T. aestivum*	0.00 a	0.00 a	0.00 a	0.10 b	0.03 a	0.01 a
Yaroslav emmer *T. dicoccum*	0.00 a	0.00 a	0.01 a	0.00 a	0.00 a	0.00 a

Table 5. The analysis of variance of the coefficient of parentage for spring durum cultivars released in the Russian Federation between 1929–2003 by six institutes. A * indicates significance at $P < 0.001$.

Source	SS	DF	MS	F
General	4.309	165		
Institutes	0.762	5	0.1525	6.879 *
Error	3.547	160	0.0222	

kept. Cultivars developed at Krasnyj Kut Breeding Station and the Siberian Agricultural Research Institute have the least similarity and, thus, higher diversity. An intermediate position is occupied by cultivars from the Samara, South-East, and Central Chernozem Agricultural Research Institutes. The diversity of cultivars from these breeding centers is estimated by approximately identical average coefficient of parentage. Cultivars developed at the Samara and Saratov Agricultural Research Institutes have the greatest Shannon diversity index and those from the Ukrainian Research Institute the least (Table 6).

Table 6. Average coefficient of parentage in sets of spring durum cultivars from various breeding institutes. Mean coefficients of parentage (R) followed by different letters are significantly different at $P = 0.05$ by the Duncan range test. N is the number of cultivars released between 1929–2003.

Institute	R	N	Shannon diversity index
Ukrainian Research Institute of Plant Production	0.30 c	7	1.89
Krasnyj Kut Breeding Station	0.07 a	7	2.13
Siberian Agricultural Research Institute	0.07 a	6	2.23
Samara Agricultural Research Institute	0.17 b	7	2.32
Agricultural Research Institute for South-East	0.13 ab	13	2.31
Agricultural Research Institute for Central Chernozem Region	0.15 ab	5	2.15

Reference.
Martynov SP and Dobrotvorskaya TV. 2000. A study of genetic diversity in wheat using the Genetic Resources Information and Analysis System GRIS. Rus J Genet **36**:195-202.

ITEMS FROM THE REPUBLIC OF SOUTH AFRICA

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Preharvest sprouting and falling number.

M. Craven.

Preharvest sprouting occurs when physiological mature wheat starts to sprout when still in the field due to heavy rain experienced during the harvest season. Although all of the current cultivated areas of the Free State are subject to PHS, the eastern Free State generally suffers the most from this phenomenon. Because sprouted wheat negatively effects the falling number, these two factors are closely linked.

The main focus of the PHS program is the routine evaluation of all current cultivars, especially newly released cultivars, for their inherent PHS resistance. The tendency of a cultivar to sprout under favorable conditions is determined with the use of a rain simulator. Approximately 3,000 wheat spikes were evaluated for this purpose during the 2003–04 season. In addition, ARC-Small Grain Institute breeding material is evaluated for PHS resistance to ensure that cultivars with poor PHS resistance are not released. During the past season, nearly 12,000 wheat spikes were evaluated for this purpose. A separate breeding program also was initiated with PHS resistance as the main focus. We hope that in the future, protein gels will simplify this task.

Because PHS is not the only factor that influences falling number (FN) of wheat, the FN project is currently focussing on factors that might influence FN, and the possibility of managing these factors to allow for the optimum FN to be obtained within a specified season. The effect of high kernel-moisture content, fertilizer applications, and the possibility of glyphosate treatments to enhance the drying period of wheat are currently long-term projects running within the FN program.

As of April 2004, a new project will be initiated at the University of the Free State to investigate the effect of frost damage at early grain-filling stage on various quality parameters. The effect of such treatments on the FN will also be investigated. We hope that this type of research will not only supply the producer with answers to unexplained low FN, but also will help to manage FN better.

Wheat production in South Africa.

A. Barnard, W.M. Otto, and T. Walsh.

Three production systems dominate in South Africa, dryland conditions in both summer and winter rainfall regions and under irrigation on a countrywide basis. Almost 50 % of the South African wheat production is accounted for by cultivation under dryland conditions in the Summer Rainfall Region, the Winter Rainfall Region and under irrigation accounting for the remaining production in South Africa. A national, cultivar-evaluation program is conducted each year at the Small Grain Institute, which entails the evaluation and characterization of all newly classified and released wheat cultivars of all seed companies on an objective and scientific basis.

The objectives of this program are mainly to characterize cultivars in terms of yield performance and yield stability, hectoliter mass, falling number, protein content, and other parameters grain quality, over environments and years. Another major objective is to compare cultivars in terms of agronomic characteristics, such as growth period, straw strength, shattering, and yield components and to make reliable and scientifically sound recommendations to producers and others for all production regions of South Africa.

The results of the program enable producers, including small-scale farmers, to make well-informed production decisions in terms of cultivar choice. Cultivar choice is a critical production decision that will greatly affect the profitability of the producer's enterprise.

Summer Rainfall Region.

Dryland production. Almost half of the South African wheat production is accounted for by cultivation under dryland conditions in the Summer Rainfall Region. Due to the large variation in climatic conditions and soil types existing in this region, wheat production is very challenging. Not only are good cultivation and management practices essential for successful wheat production, but also the correct cultivar choice. The dryland production area is divided mainly into four homogenous areas where different cultivars, mainly winter and intermediate types, are planted. Cultivar evaluation trials were planted at 17 sites throughout the Western, Central, and Eastern Free States and parts of Mpumalanga. The trials were successful and were reported. Twenty entries were included in the trials, of which seven were from the Small Grain Institute, seven from Monsanto, and six from PANNAR.

Production under irrigation. Wheat produced under irrigation amounts to about 20 % of the total wheat production of South Africa and has a stabilizing influence on the total production. Currently, six major irrigation regions exist, although irrigation farming is expanding into new production regions.

Spring wheat cultivars are planted mainly in a total of 60 evaluation trials at 31 localities in the different irrigation areas. Entries in these trials were from the Small Grain Institute (7) and Monsanto (5). Three advanced breeding lines also were included. Analyses of variance, AMMI analysis, and biplots are used in the interpretation of results and in identifying cultivar adaptation and stability in the different production regions. Results from these trials are available in a detailed report.

Winter Rainfall Region.

Two wheat producing areas are in the Winter Rainfall Region

The Swartland area stretches from Durbanville in the south to the Sandveld area around Elandsbaai in the north and from Saldana Bay in the west to the mountain ranges in the east.

The Rûens or South Coast area, stretches from Botrivier in the west to the Albertina-district in the east and from Aghullas in the south to the Langeberg mountain range north of Greyton through to Riversdal.

The Winter Rainfall Region is well suited to the production of spring wheat cultivars, which do not require the same amount of cold to break their dormancy as that of the winter wheat cultivars grown in the rest of South Africa. Cultivar choice in the Winter Rainfall Region is of extreme importance due to the varied climatic differences between cultivation areas. The cultivars available differ in their yield reaction to the changing yield potential conditions that exist in the Winter Rainfall Region. Other important factors that also have to be taken into consideration are grain quality, hectoliter mass, and disease susceptibility.

The Western Cape experienced extreme weather pattern differences during the 2003 season resulting in a significant reduction in overall yield for the area. The Swartland area of the Western Cape had the lowest rainfall in decades, with the average rainfall for the period April to October being 27.5 % less than the long-term average for the area. The Rûens area, although wetter than the Swartland, recorded an averaged of 2.4 % less rain than the long-term average for the area.

Because of drought conditions in the Swartland during the 2003 production period, many lands were not cultivated or planted. Land that was planted yielded 50 % less on the average than that of a normal season. Weed and disease control was cut to the minimum. The result was a visible increase in weeds and also a decrease in grain quality due to low hectoliter mass.

The Cultivar Evaluation Program in the Winter Rainfall Region is run jointly by the Small Grain Institute and The Directorate of Agriculture of the Western Cape. The program consists of 13 sites in the Swartland and 13 sites in the Rûens, with 14 cultivars included in the trials. The cultivars, from ARC-Small Grain Institute, Monsanto, and PANNAR,

are annually tested for yield potential, quality, disease resistance, and adaptability. However, because of the drought conditions that prevailed in the Swartland this past season, only three trial sites were harvested and appraised. These three sites were all located in the Sandveld area of the Swartland. The trials in the Rûens-area were harvested and reported.

Verification of cultivars suitable for production in resource limited agriculture.

S. Ramburan.

As part of the National Cultivar Evaluation Programme, the Small Grain Institute has introduced a related program in 2003 that involves the screening of wheat cultivars suitable for production in resource limited agriculture. Differences in production practices and resources of small-scale enterprises in comparison to commercial situations necessitated the introduction of cultivar-evaluation work in the resource limited areas of the country. A large proportion of resource-limited farmers in the major wheat producing regions of South Africa have the potential for commercialization and correct cultivar choice is sure to assist them in reaching this ultimate goal.

In 2003, cultivar-evaluation trials were planted at various small-scale farms that were representative of specific wheat-producing regions. A total of four dryland trials (15 cultivars) and three irrigation trials (16 cultivars) were planted. Cultivars originated from three different institutes, Small Grain Institute, Pannar, and Monsanto. During the season, the adaptability of the cultivars to the production environments were evaluated through observations of emergence problems, growth period, and disease damage. Statistical analyses were used to determine the yield and quality performance of the cultivars in the different environments.

The data obtained from the project will be used to ultimately characterize the different cultivars in terms of their suitability for production in different resource-limited areas. These results, together with those expected in 2004 and 2005, will eventually be used to assist small-scale wheat producers with reliable recommendations that are based on applicable scientific research.

Pest control research at the Small Grain Institute.

G.J. Prinsloo.

Research on the control of different wheat insect pests is continuing. Our focus is on developing and using different kinds of alternate control methods. Besides the major use of plant resistance to control the RWA, other control agents like predators, parasitoids, entomopathogenic fungi, and the usefulness of plant volatiles are researched in collaboration with national and international partners. All these control options are being used in a integrated control program which, together with a diverse environment, are aimed at the prevention of the development of a resistant-breaking biotype of RWA. At the same time, these environmentally safe methods also are being used to control the minor insect pests of wheat in the country.

Fusarium head blight.

B. Khubeka.

Fusarium head blight has become a serious disease of both wheat and barley in South Africa. Regular yield losses varying from 5 to 40 % on wheat produced under center-pivot irrigation have been recorded. Research on the evaluation of fungicides and different cultivars is conducted in South Africa to control FHB.

New stripe rust virulence detected on Yr1 in South Africa.

J.S. Komen.

Since the first outbreak of *Puccinia striiformis* f. sp. *tritici* in 1996, wheat fields were surveyed periodically by researchers of the Small Grain Institute to monitor the occurrence, development, and distribution of stripe rust in South Africa. Disease nurseries are planted annually in all the important wheat-production areas of South Africa. Surveys also are carried out in Lesotho were wheat is grown in the summer. Samples were taken at the end of February from the disease nursery in Lesotho and pure isolates were inoculated on the world and European differentials. New virulence for *Yr1* was detected in Lesotho during stripe rust surveys. The new pathotype has shown no new virulence on the commercially cultivars planted in the production areas of South Africa. The new pathotype 7E22A differs only from pathotype 6E22A (found in 1997) by its virulence to *Yr1*.

Small Grain Institute Laboratories: Seed Testing Laboratory.

H. Hatting.

Seed plays a vital role in the potential crop yield of each small grain producer. Small grain seed must comply with legal requirements with regard to the purity and germination percentage before it can be marketed. The Small Grain Institute has a registered Seed Testing Laboratory in which international methodology (ISTA (International Seed Testing Association) methods) is used to determine the quality characteristics of seed. Germination and purity testing over the past year resulted in 456 analyses, in which the quality of each seed lot was tested to ensure that poor quality seed would not be planted. The laboratory provides a unique service. Having the infrastructure and experience, seed analyses are conducted objectively on a commercial and need-driven basis for the seed industry.

The laboratory was visited by several schools. This work contributes favorably to the income of the Seed Testing Laboratory. The services are client-specific and extended the commercial services of the laboratory.

Small Grain Institute Laboratories: Wheat Quality Laboratory.

C.W. Miles.

The Wheat Quality Laboratory plays an integral part in the breeding process and accurate and reliable data for researchers must be ensured. To accomplish accuracy and reliability, the laboratory takes part in ring tests sent out monthly by Sasko and quarterly by the South African Grain Laboratory.

During the past year, a total of 66,083 analyses were performed for researchers at the Small Grain Institute and 5,003 analyses were performed for external clients.

Small Grain Institute Laboratories: Soil Analyses Laboratory.

M.H. Visser.

The laboratory experienced a very prosperous 2003–04. In spite of a dry season and the fact that farmers in the laboratories operational area planted 25 % less wheat, the external income was increased by five percent.

During the year the following activities took place:

Lientjie Visser received training at different USA laboratories,
Marie Potgieter attended an ICP-course at the Pretoria Technicon,
a new Compact Titrator was purchased,
the database was upgraded with information on rural areas in the Eastern Cape and Thaba ‘Nchu, and
laboratory personnel attended two meetings of Agri-LASA, the national control scheme of South Africa’s laboratories.

The main objective of the laboratory will always be to provide clients with accurate and reliable results, upon which they can make the necessary management decisions.

Personnel.

Ms. Una Aucamp has resigned as a breeder and Ms. Rachel Oelofse was appointed to replace Ms. Anri Barnard. Anelizwa Makhathini joined the Small Grain Institute as a research technician in Plant Breeding. Dr. Hussein Shimelis has been appointed temporarily at Plant Protection as a rust expert. Iona Basdew replaced Karen Naudé to handle the Karnal bunt laboratory, washing facility, and take-all research. Bongani Kubheka replaced Khaya Ntushelo as a researcher on *Fusarium*. Cedric Baloyi, Lucas Serage, Nora Mokoka, and Tshepo Maeko were appointed at Soil Management as researchers. Kenneth Mokoena joined Soil Management as a research technician.

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A-M. Botha, M.T. Matsioloko, F.B. du Preez, L. van Eck, K. Muller, R. van Zyl, A.C. Laubser, E. Swanepoel, Z.A. Swanevelder, and R. Walters.

Profiling of cDNA-AFLP derived transcripts in elucidated in response to Russian wheat aphid feeding.

M.T. Matsioloko, L. Van Eck, R. Walters, and A-M. Botha.

The RWA is a major factor hampering the production of wheat. In the present study, wheat NILs Tugela DN (*Dn1*; SA1684/*Tugela), Tugela *Dn2* (SA2199/*5 Tugela), Tugela *Dn5* (SA463/*5 Tugela), and Tugela were employed to study differential gene expression induced by aphid infestation using cDNA–AFLP analysis. cDNA was synthesized using mRNA extracted from leaf material collected at 0, 1, 2, 6, 12, 24, 48, and 120 hours post infestation (HPI) with RWA. cDNA–AFLP profiles were obtained after analysis on a LICOR automated analyzer. Differentially expressed, transcript-derived fragments (TDFs) were identified following an *in silico* subtraction of the measured band intensities of Tugela from the Tugela DN. The data were further analyzed using Hierarchical Cluster Analysis and Spearman Rank Correlation. Subclusters of TDFs (Table 1) indicated genes putatively involved in conferring resistance to the RWA.

Table 1. Subclusters of differentially expressed transcript-derived fragment expression patterns as generated by a Hierarchical Cluster analysis for genes putatively involved in conferring resistance to the Russian wheat aphid. 0 = no expression, — = low expression or down regulation, and + = high expression or up regulation.

Subcluster Number	Hours post infection							
	0	1	2	6	12	24	48	120
1	—	+	0	0	—	+	—	+
2	+	+	—	0	—	—	—	+
3	—	+	+	—	0	0	+	—
4	—	0	0	—	+	+	+	—
5	—	+	—	—	+	+	—	—
6	—	+	—	—	—	—	+	+
7	—	—	+	+	—	—	—	—
8	+	+	+	—	—	—	—	—
9	+	—	—	+	+	—	+	—
10	+	0	—	—	—	+	+	+
11	—	—	+	—	—	+	+	+
12	0	—	—	—	—	+	+	+
13	+	+	—	—	—	+	+	—
14	—	—	0	—	+	+	+	+
15	—	+	+	+	—	—	—	—

Tracking nucleotide-binding-site-leucine-rich-repeat resistance gene analogs in the wheat genome complex.

F.B. du Preez and A-M. Botha.

Our current research focuses on the identification and classification of R-gene candidates from the wheat genome; more specifically on the NBS–LRR gene family for which a large number of members and their avirulence complements have been cloned across multiple plant genera. Using initial Position-Specific Iterated Blast searches (PSI-Blast), many putative members of this gene family for species of the *Triticeae* tribe were identified and used to build a Hidden Markov Model (HMM) of the nucleotide-binding-site domain of this gene family. Using this model, many high-scoring, EST sequences generated by among others the International Triticeae EST Cooperative (ITEC) were obtained.

Phylogenetic analysis of the gene family members obtained for wheat and its putative genome-donor species was performed. A comparative analysis between the wheat group, barley, rice, and *Arabidopsis* confirmed previous observations with regard to the domain structure distribution observed over large taxonomic groups. NBS–LRR RGA sequences have great utility in marker design for mapping the position of localized clusters of this resistance-gene family and for identification of candidate open-reading frames in mapped R-gene containing regions.

Genetic mapping of the Russian wheat aphid resistance genes Dn1, Dn2, and Dn5.

K. Muller, A.C. Laubser, E. Swanepoel, and A-M. Botha.

Two thousand wheat ESTs, including 156 wheat NBS–LRR sequences were obtained using degenerate primer sets designed from the consensus NBS region from other genome studies (e.g., *Arabidopsis* and rice), RACE–PCR, suppression subtractive hybridization (SSH), and cDNA libraries. Previously, we confirmed the cosegregation of a SSH clone, *AMO00SSHL1* (GenBank accession # AF4446141.1; e^{-125}) and NBS-RGA2 (368 bp; GenBank accession # AF326781; 7e-13; NBS-RGA2) with the RWA resistance gene *Dn1* at a linkage distances of 7.41 cM and 3.15 cM, respectively. We presently are constructing a framework map using SSRs and AFLP analysis, and a $F_{2/3}$ segregating population composed of 184 individuals that derived from a 'Tugela/Tugela DN' cross in an effort to map all the ESTs obtained through our study. The genetic map will provide information to be used in the physical mapping of these genes using BAC libraries.

Characterization of proteins expressed in response to Russian wheat aphid infestation.

R. van Zyl, Z.A. Swanevelder, and A-M. Botha.

RWA feeding causes the induction and down regulation of proteins as well as the appearance of novel proteins, as the plant attempts to defend itself against attack. The exact function of these proteins is unknown, but they appear to be involved in the defense of the plant against aphid feeding. RWA-induced proteins are found in the apoplast (along the stylet route) and it has been shown that aphid feeding on resistant plants turn to nonphloem feeding to survive. Induction of proteins was confirmed during RWA infestation of the resistant cultivar Tugela DN.

Two-dimensional gel electrophoresis has shown that bands obtained from SDS–PAGE analysis represent more than one protein. This study shows an induction of five groups of protein, which correspond to seven proteins. The first and second induced proteins (~ 36 and 26 kDa) could possibly be related to the PR-proteins, β-1,3-glucanases and chitinases. These enzymes have sizes that correspond to those of the induced PR proteins. We previously reported that RWA infestation induced β-1,3-glucanase isozymes having pI values ranging from 3.6 to 9.3, whereas feeding results in the expression of one chitinase isozyme with a pI of 5.5. Group-3 proteins are not serologically identified. Occurring close to this group was three induced proteins (all ~ 20 kDa) and two induced proteins (< 14 kDa). These proteins are not similar to the group-4 proteins. Protein sequence analysis of induced proteins would help clarify the exact nature of these proteins and their role in plant defense and, thus, the analysis of these induced proteins using MALDITOFF is under way.

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Triticale breeding.

The breeding program was continued and promising new lines were selected. Of the six commercial cultivars, USGEN 19, Rex, Kiewiet, Bacchus, Tobie, and Ibis, Kiewiet has become susceptible to stem rust, whereas Rex and Bacchus have shown moderate susceptibility. Results of the 2003 trials have shown that, on average, the better grain yielders, Bacchus and Tobie, out-yielded the best wheat cultivars by 27 %. Plant breeder's rights were obtained for a new rye cultivar Duiker.

Wheat recurrent mass selection.

A commercial-scale, recurrent-selection program is being established. Following each selection cycle (4 years), the *Ms3* male-sterility gene and hydroponic culture of cut tillers are being used to effect cross-pollination of male and female spikes. Female plants destined for crosses are selected as F_1 seedlings, however, male parents are field tested and are not used in crosses until the F_7. We managed to advance F_1 male families to the F_6 in only two seasons (2002–03), making use of single-seed descent and off-season planting. Following stringent field selection (single spikes) among and within F_4 families for leaf, stem, and stripe rust resistance during the winter of 2003, about 450 F_5 rows were grown during the summer (2003–04). These will be evaluated as unreplicated F_6 field plots in the winter of 2004. From these nurseries, the first set of male parents will be selected for use in crosses in the fourth year (2005). The size of the breeding population will gradually be expanded in the coming years. In 2004, an attempt will be made to introduce MAS on a limited scale.

Genetic studies.

In a program aimed at the transfer of leaf, stem, and stripe rust resistance genes from wild relatives, we found two closely linked, leaf and stripe rust-resistance genes from *T. turgidum* subsp. *dicoccoides* were inserted on chromosome arm 6BS. Leaf rust resistance from *Ae. kotschyi* could be translocated from a group-2 chromosome addition to a wheat chromosome, probably 2D, utilizing the tendency of unpaired chromosomes to undergo centric break and fusion translocations. Aneuploid analyses to map genes from *Ae. sharonensis* and *Ae. peregrina* were continued. An attempt to shorten chromosome segments derived from *Ae. speltoides* through disruption of meiotic chromosome pairing was continued, and an attempt to induce translocation of target genes from unknown group-3 chromosomes of *Ae. biuncialis* and *Ae. caudata* to wheat.

We confirmed that RWA-resistance gene, *Dn5,* is located on chromosome arm 7DL of wheat. Telosomes 7DL with and without *Dn5* could be recovered and confirmed with molecular markers. A DH mapping population derived from the cross 'PI294994/Chinese Spring' is now being characterized in an attempt to relate *Dn5* to known markers on 7DL.

A recombined *Lr19* translocation, *Lr19*-149-299, was used in an attempt to shorten it still further through use of *ph1b*-induced homoeologous pairing. Testcross progeny are being screened specifically for the absence of the segregation distortion gene, *Sd2*.

In a program aiming to transfer salt tolerance from *Th. distichum* to triticale, chromosomes IIJ_1^d, $IIIJ_1^d$, IVJ_1^d, VJ_1^d, and $VIIJ_1^d$ were putatively identified as being involved in its expression. An attempt is being made to develop disomic addition lines of each target chromosome in triticale. Additions were recovered for $IIIJ_1^d$ and VJ_1^d. The additions were crossed to triticale monosomics for the A, B, and R genomes of the corresponding homoeologous groups. For each cross, double monosomic lines were selected and testcrossed with triticale. The testcross F_1 is being screened (GISH) for centric breaks and fusion translocations involving triticale and *Thinopyrum* chromosomes. An attempt to develop SCAR markers for the critical *Thinopyrum* chromosomes has produced three markers, specific for IIJ_1^d and $IIIJ_1^d$. Backcrosses to develop the remaining additions are being continued.

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ITEMS FROM SPAIN

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Changes in peroxidase gene expression in response to Heterodera avenae infection in a wheat/ Aegilops ventricosa introgression line carrying the resistance gene Cre2.

The quasi-dominant, resistance gene *Cre*2 in H-93-8 wheat–*Ae. ventricosa* introgression line confers a high level of resistance to several European populations of cereal cyst nematode *H. avenae*, including the Spanish pathotype Ha71 (Delibes et al. 1993). An incompatible interaction between line H-93-8 and the nematode are determined by hypersensitive response, which is frequently preceded by the formation of active oxygen species (AOS). Plants possess both enzymatic and nonenzymatic antioxidant defence systems to counteract AOS under stress conditions. The antioxidant enzymes include peroxidase (PER, E.C.1.11.1.7), esterase (EST, E.C. 3.1.1.2), and superoxide dismutase (SOD, E.C.

1.15.1.1). Isoelectricfocusing isoenzyme analysis, 4 and 7 days-after-infection, revealed that PER, EST, and SOD activities increased in the resistant line H-93-8 in comparison with the susceptible control (Andrés et al. 2001; Delibes et al. 2002; Montes et al. 2003). Nematode infection preferentially enhanced the activity of PER system, with a notable increase in cationic and anionic isozymes, as described in other incompatible reactions (Zacheo and Bleve-Zacheo 1993; Montes et al. 2004) and lent further support to the participation of lignification in the nonhost resistance.

In this work, PER mRNA transcripts, on infected and uninfected H-93-8 and a susceptible control, have been analyzed by Northern analyses using a PER probe obtained by PCR. RNAs were extracted from leaves and roots 4 and 7 days-after-infection. In the resistant line, 7 days postinoculation, a significant increase of peroxidase mRNA level was observed in roots at the nematode feeding site. No differential induction was detected in the susceptible control. Foliar mRNA levels were not affected by inoculation in any genotype. The root specific expression pattern of PER genes was determined by RT–PCR analysis, which the DNA second-strand was synthesized using a primer specific of PER. PCR products were cloned and sequenced, showing a high similarity to previously described cereal peroxidase genes. After a second round of RT–PCR with primers designed starting from the first RT–PCR sequences, differential mRNA transcripts from the nematode feeding site were obtained. Among the induced products, we found certain PER mRNA species that shown a C-terminal extension, which appears to target for vacuolar import as described in *A. thaliana* (Welinder et al. 2002). Peroxidase activity pattern and transcript accumulation profile suggests a role for peroxidase in resistance, probably in cell wall cross-linking.

In parallel to the described study, we are developing a new type of molecular markers for *Cre*2 and other resistance genes reported by our group in wheat. The *H. avenae* resistance from line H-93-8 was introduced into commercial wheat by backcrossing and selection. To assist the selection of resistance conferred by *Cre*2, we are currently searching markers based on LRR sequences described as major determinants of the specificity of resistance genes in plants. We are trying to obtain these markers by PCR using primers designed from NBS domain of resistance genes and transposons commonly inserted near rich gene regions. The novel markers will be scored as codominant SNP-based PCR markers as reported by Moreno-Vázquez et al. (2003).

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ITEMS FROM TURKEY

CIMMYT
P.K. 39 Emek, 06511 Ankara, Turkey.

A.R. Hede, H.J. Braun, J. Nicol, and B. Akin.

THE MINISTRY OF AGRICULTURE AND RURAL AFFAIRS
Turkey.

M. Keser, N. Bolat, N. Colak, H. Ekiz, S. Taner, S. Ceri, F. Partigoc, L. Cetin, S. Albustan, F. E. Donmez, F. Dusunceli, S. Yazar, I. Ozseven, I. Ozturk, and T. Yildirim.

ICARDA
Aleppo, Syria.

M. Mousaad and A. Yahyaoui.

In 1986, the Turkish Ministry of Agriculture, CIMMYT, and ICARDA joined forces to conduct research on winter wheat through the International Winter Wheat Improvement Program (IWWIP). The two main objectives of the program are to develop broadly adapted, disease-resistant, high-yielding winter wheat germ plasm for the winter and facultative wheat growing areas in Central and West Asia and North Africa (CWANA) and to help facilitate germ plasm exchange among the winter wheat breeding programs around the world.

About 31 x 10^6 hectares of the 103 x 10^6 hectares of wheat in low-income countries is facultative or winter wheat, of which 16.5 x 10^6 hectares are grown in Central and West Asia and North Africa, 13 x 10^6 hectares in China, and 1 x 10^6 hectares in South America, north Africa, and North Korea.

International nurseries.

Germ plasm exchange and evaluation is facilitated through the yearly shipment of various international nurseries to a number of collaborators around the world. The nurseries are evaluated for grain yield potential, resistance to various abiotic and biotic diseases, and quality. Data returned to IWWIP is utilized in the continuous breeding program. Germ plasm selected by coöperators either is being utilized as parents in their breeding programs or as direct cultivar releases. The following nurseries are being distributed from the IWWIP program, and we would like to use the opportunity to thank old collaborators and invite new collaborators to enter this network of wheat germ plasm exchange.

The Facultative and Winter Wheat Observation Nursery (FAWWON). The FAWWON has served as the main vehicle for facilitating germ plasm exchange among winter wheat programs. This nursery consists of lines developed by the IWWIP program and cultivars submitted by national programs, university programs, or private companies from countries in the CWANA, western and eastern Europe, China, South America, and the U.S.A.

Most lines developed by the IWWIP program show a good level of resistance to stripe rust, although many of the lines submitted by programs where stripe rust is not a problem are highly susceptible. However, because many of these lines have highly favorable characteristics, sharing such information with all coöperators is important in order to utilize more efficiently these lines in breeding programs. Otherwise, they great risk of being discarded by breeders due to stripe rust susceptibility. We decided, therefore, to discontinue the FAWWON as it is and it was not distributed to coöoperators during the 2003–04 season. Recognizing the great importance of FAWWON as a germ plasm exchange tool however, we will start to distribute it again starting from the 2004–05 season. Only cultivars that are accompanied

by a description of desirable traits will be included, assuring a maximum utilization of the germ plasm. The FAWWON nursery normally consists of between 150–200 entries (15 g/entry) and is distributed to more than 100 coöperators.

Wheat Observation Nursery for Irrigated Areas and Wheat Observation Nursery for Semi-Arid Areas (WON-IRR and WON-SA). The Wheat Observation Nursery for Irrigated Areas and Wheat Observation Nursery for Semi-Arid Areas consist of lines developed by the IWWIP program. These lines have been selected based on performance in yield trials in Turkey and Syria and based on their resistance to diseases, in particular to yellow rust and common bunt. The WON-IRR and WON-SA is targeted for wheat-growing areas in central and west Asia.

The WON-IRR and WON-SA nurseries normally consist of between 100–125 entries (15 g/entry) and are distributed to 40–50 coöperators.

Elite Yield Trial for Irrigated Areas and the Elite Yield Trial for Rainfed Areas (EYT-IRR and EYT-RF). The Elite Yield Trial for Irrigated Areas and the Elite Yield Trial for Rainfed Areas consisted each of 25 entries with three replications with 160–180 g seed/replication. The entries were selected from the previous WON-IRR and WON-SA respectively. The EYT-IRR and EYT-RF nurseries are distributed to 40–50 coöperators.

Eurasian Winter Wheat Yield Nursery (EURAWWYN). The Eurasian Winter Wheat Yield Nursery (EURAWWYN), previously WWEERYT (Winter Wheat East European Yield Trial) consists of approximately 50–60 elite wheat cultivars ready for release and developed by breeding programs in eastern Europe, the Russian Federation, central and west Asia, and the U.S.A. Coöperators submit seed for multiplication to Turkey, from where the nursery is shipped the following year. Access to this nursery is restricted. Only coöperators who have signed the MTA, which follows the Code of Ethics for Wheat Workers, receive the nursery. The steering committee for the WWEERYT has to approve request of new coöperators.

The objectives of the network are to

provide elite wheat cultivars for immediate release to weaker NARS,
provide rapid seed multiplication of selected lines (fast seed increases for each entry, up to 3 kg seed are distributed, to allow also large scale testing already in year one),
facilitate germ plasm exchange of elite winter wheat lines among participating institutions, and
improve communication and knowledge exchange through scientific meetings.

Germ plasm development and cultivar releases.

Since 1994, 27 cultivars from the IWWIP program have been released in Afghanistan, Argentina, Georgia, Iran, Kyrgyzstan, Turkey, and Uzbekistan (Table 1, p. 171). Some cultivars have been released under different names in different countries, demonstrating the broad adaptability of IWWIP germ plasm. Cultivars originating from the same cross are marked with identical number in Table 1. For example, the cross 'YMH/TOB//MCD/3/LIRA' was released in Afghanistan in 1994 under the name PAMIR94, in Turkey in 1997 under the name KINACHI97, in Uzbekistan in 2002 under the name DOSTLIK, and is included in registration trials in Iran. Most of these cultivars have been released after having been introduced through one of the international nurseries distributed through the IWWIP program, demonstrating the great importance of international coöperation and germ plasm exchange for crop development.

Fourteen of the 27 cultivars were selected from populations that had been tested at different locations, e.g., the cultivar Djamin was selected from a cross made in Mexico, the F_2 and F_3 were tested in the U.S. (Oregon), and the F_4– F_8 were selected in Turkey, emphasizing the power of international germ plasm exchange.

Table 1. International Winter Wheat Improvement Program (IWWIP)-derived cultivars registered in central and west Asia and north Africa since 1994. Cultivars with identical numbers ([1–5]) originate from the same cross but are released under different names in several countries.

No.	Country	Year	Cultivar name	Cross	Pedigree
1	Afghanistan	1994	PAMIR94 [1]	YMH/TOB//MCD/3/LIRA	-7M-0M-8M-1M-3WM -0AFG
2	Afghanistan	1996	GUL96 [2]	ID800994.W/VEE	-2AP-2AP-1AP-1AP-0AP
3	Afghanistan	1996	RANA96	CA8055/6/PATO(R)/CAL/3/7C/BB// CNO/5/CAL//CNO/SN64/74/CNO// NAD/CH	-2AP-2AP-1AP-1AP-0AP
4	Afghanistan	2002	SOLH02 [3]	OK82282//BOW/NKT	-0YC-0YC-4YC-0YC
5	Argentina	1996	Buck Oportuno [4]	PI?FUNO*2??VLD/3/CO723595	-9H-4M-3WM-0WM
6	Georgia	2002	Mtshetskaya [1]	TAST/SPRW//ZAR	-3AP-1AP-2AP-0AP-1AP -0AP
7	Iran	1996	Zarrin	NAI60/Heine VII//BUC/3/F59.71/GHK	
8	Iran	2000	C73-5	SP/MCD//CAMA/3/NZT	-17H-4H-1H-0H
9	Kyrghyzstan	2002	Djamin	NS55-58/VEE	-08H-1P-0TK-0TK-0TK -10YC-0YC
10	Kyrghyzstan	2004	Azibrosh [3]	OK82282//BOW/NKT	-0YC-0YC-0YC-2YC-0YC
11	Kyrghyzstan	2004	Zubkov	1D13/MLT//KAUZ	-2H-0PE-0YC-3YC-0YC
12	Turkey	1991	GUN91	F35.70/Mo	-1A-1A-1A-0A
13	Turkey	1995	SULTAN95 [5]	AGRI/NAC	-2H-1H-3P-0P-5M-3WM -0WM
14	Turkey	1997	KINACI97 [1]	YMH/TOB//MCD/3/LIRA	-7M-0M-8M-1M-3WM -0WM-4WM-2WM-0WM
15	Turkey	1998	YILDIZ 98	1744/P101//MAYA/3/MUS/PRM//MAYA/A	-1P-0TE-23YA-0E
16	Turkey	1999	GOKSU99 [5]	AGRI/NAC	
17	Turkey	2000	Cetinel 2000	MLC/4/VPM/MOS95//HILL/3/SPN	-6H-0YC-0R-1YC-0YC-0E
18	Turkey	2001	Alpaslan	TX69A509-2//BBY2/FOX	-2YC-0YC
19	Turkey	2001	ALPU2001 [2]	ID800994.W/VEE	-1WM-0WM-0SE-0YC -HRC*-6YC-0YC
20	Turkey	2001	IZGI	CA8055/KUTLUK	-0AP-0YC-0YC-1YC-0YC
21	Turkey	2001	Sonmez	ATIA1	-0T-0YC-0YC-5YC-0YC
22	Turkey	2002	BAGCI2002	HN7/OROFEN//BJN8/3/SERI82 /4/74CB462/Trapper//VONA	-0SE-2YC-0YC-1YC
23	Turkey	2002	Daphan	00477	
24	Turkey	2002	Nenehatun	ND/P101/Blueboy	-0P-1P-2P-0H
25	Turkey	2002	Sakin [4]	PI/FUNO*2//VLD/3/CO723595	-4WM-0Wm-3N-1N-0N
26	Turkey	2002	Soyer	ATAY/GALVEZ	-0SE-4YC-0YC-2YC-0YC -1YC
27	Turkey	2002	Yildirim [2]	ID800994.W/VEE	-1WM-0WM-0SE
28	Uzbekistan	2002	Dostlik [1]	YMH/TOB//MCD/3/LIRA	-7M-0M-8M-1M-3WM-0WM-4WM-2WM-0WM

Research on root rots and nematodes — Progress update of Turkey–CIMMYT collaboration from 2003.

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Since 1998, the Ministry of Agriculture and Rural Affairs in Turkey (MARA) in collaboration with CIMMYT staff based in Turkey have initiated two key National/International projects. One of these is on cereal nematodes and the other on cereal root rots. These projects cover a range of research areas including

i) surveys,
ii) economic importance and population dynamics,
iii) identification of sources of resistance, and
iv) control methods with an emphasis on plant genetic resistance.

Below is a brief update on progress of some of these research objectives since our last report (Ann Wheat Newslet 2003, **49**:147-159). We very much encourage anyone interested in collaborating with our program to contact us.

Nematode work: Yield loss caused by the cereal cyst nematode. Two separate trial locations of rainfed winter wheat production areas in Turkey included one on a farmer's field in Cifteler and the other a MARA research station in Haymana. Nematode yield-loss trials are currently in their second year. The key objective of these trials is to understand the yield loss and population dynamics of the CCN *(H. filipjevi*) on the common cereal cultivars on the Central Anatolian Plateau of Turkey.

The trials consisted of seven replicated yield plots with and without the application of the nematicide Temik (active ingredient Aldicarb). Twelve different cereal cultivars were investigated; three spring wheats from Australia with two known CCN-resistance genes, six representative Turkish Winter wheats, one Turkish barley, and one Turkish triticale. The statistical design was a split-plot RCBD with pair-wise cultivars with and without application of nematicide. At the start and end of the season, the initial density of *H. filipjevi* (and other nematodes) was recorded by intensively coring 15 soil samples/ plot and subsequent extraction from one composite sample/plot. Yield and other plant parameters were taken as appropriate.

Table 2. Percent yield (t/ha) increase of Turkish and Australian cereals with the application of the nematicide Temik im cereal cyst nematode-infested fields in two locations (Haymana and Cifteler) of the Central Anatolian Plateau of Turkey during the 2002–03 season (preliminary data). Data are significant at 0.05. *Cre1* and *Cre8* are known sources of resistance to the cereal cyst nematode *H. avenae* pathotype *Ha13*. PN indicates resistance to the root-lesion nematode *P. neglectus*.

Cultivar	Cereal type and country of origin	Haymana	Cifteler
Kalayci – 97	Barley (Turkey)	7	8
Silverstar	Spring wheat *Cre1* (Australia)	38	36
Gerek – 79	Winter wheat (Turkey)	39	20
Karma – 2000	Triticale (Turkey)	41	20
Bezostaya – 1	Winter wheat (Turkey)	41	48
Bagci – 02	Winter wheat (Turkey)	41	21
Altay – 2000	Winter wheat (Turkey)	43	21
Chara	Spring wheat *PN* (Australia)	59	36
Frame	Spring wheat *Cre8* (Australia)	61	68
Kutluk – 94	Winter wheat (Turkey)	76	24
Dagdas – 94	Winter wheat (Turkey)	80	27
Gun – 91	Winter wheat (Turkey)	89	15
Average yield loss (%)		46	27

The results clearly demonstrate that CCN is economically important on rainfed winter wheat in Turkey (Table 2), with an average yield increase with Temik application of 27 % at Cifteler and 46 % in Haymana. The higher yield increase in Haymana could be explained by CCN populations three times higher than at Cifteler. Further analysis of

the data showed strong significant regression equations between yield and initial density of *H. filipjevi*, implicating this as one of the major causes of the yield loss. Other factors likely also contribute to the yield loss because Temik is known to control soil organisms other than nematodes, however, the other main soil pathogen of dryland rot rots was assessed in both trials and considered negligible.

The results indicate that the Turkish winter wheats and the triticale Karma tested suffer yield loss from CCN, however, Kalayci barley seems to offer some tolerance (limited yield loss). Preliminary data on the hosting ability of the plants by examining the multiplication rate (final population/initial population) of CCN clearly indicates that the Turkish winter wheats and the barley are susceptible (multiply the nematode), however the Australian cultivar Silverstar, with *Cre1* gene, and the triticale Karma appear to be have a level of resistance (do not multiply the nematode). Although the barley Kalayci is tolerant, it is highly susceptible from this data. This data will be further confirmed later this year after the 2nd year of trials is harvested.

This information clearly supports the need to develop integrated control strategies for CCN on the Central Anatolian Plateau of Turkey. Similar losses are likely to be experienced in the region where the nematode is known to be present and a rainfed cereal system dominates. CIMMYT and MARA Turkey continue their work on control methods with emphasis on resistance in addition to exploring other options, such as rotation and cultivation practices.

Optimization and preliminary study on Cre3 and Cre1 markers for marker-assisted selection of germ plasm to incorporate resistance.

Work in Turkey under the IWWIP in collaboration with CIMMYT–Mexico has optimized the use of two PCR markers, *Cre1* and *Cre3* from Australia. These genes offer effective resistance to a closely related nematode species, *H. avenae*, pathotype *Ha13*, and are routinely used in Australian breeding programs. *Cre1* originated from *T. aestivum*. *Cre3* is from *Ae. tauschii* and now has been introgressed into a bread wheat background by Australian researchers. These markers are highly diagnostic and, if effective against the Turkish CCN populations (*H. filipjevi* and *H. latipons*), offer an excellent tool for the integration of resistance into IWWIP and CIMMYT spring wheat germ plasm.

After marker optimization, a limited study examining 110 varied germ plasm (land races, Turkish cultivars, wild relatives, and IWWIP lines) in the IWWIP program did not find either marker present. Preliminary work indicates that *Cre1* may be effective against *H. filipjevi* and we are checking *Cre3* and other known genes of interest against cultured populations of cyst nematode species from cereals in Turkey. Subject to this confirmation, MAS using F_2 and F_4 winter wheat breeding populations will begin on a limited scale during this season. Work continues routinely at CIMMYT–Mexico incorporating both genes into spring wheat backgrounds.

Screening for resistance to the dryland root rot complex.

As reported last year (Ann Wheat Newslet 2003), after 3 years of extensive field screening for dryland root rot, we identified 31 winter wheat germ plasm lines with a good level of resistance. These materials, plus additional lines, were screened in a replicated field trial, the Root Rot Elite Line Nursery, in 2002–03 at Cumra, 40 km south of Konya, Turkey (Table 3, p. 174-175). Field observation plots were assessed by inoculating seed with a combination of root rot species (*Fusarium pseudograminearum*, *F. culmorum*, and *Bipolaris sorokinana*) comparing symptom development against noninoculated plots. Resistance is a reduction in symptom development of Fusarium crown rot, which is defined as the % white heads in inoculated versus noninoculated observation plots. A score of less than 3 indicates a degree of resistance, higher resistance is inferred by a lower score.

Six lines are not included from last years list because two have been discarded and four unfortunately were not included. An additional 26 lines have been added that include both winter wheat and spring wheat germ plasm from CIMMYT–Mexico with high yield and quality characters. These winter and spring wheat lines are now entering yield trials to assess tolerance (yield loss) and also have been extensively crossed in the IWWIP program. Similarly, this winter wheat material was incorporated into spring wheat background for CIMMYT–Mexico. Fortunately, several of the identified lines are widely grown cultivars such as Gerek and Dagdas.

Table 3. Germ plasm from replicated Root Rot Elite Line Nursery 2003 with field resistance to root rot complex under inoculated field conditions at Cumra, Turkey. Differences in cultivars are significant at 0.01; SED = 0.5. Table is continued on p. 175.

Cross	Origin [1]	Type [2]	Score [3]
KRC66/SERI/4/YMH/TOB//MCD/3/LIRA	TCI	WW	1.33
BILINMIYEN96.7	TCI	WW	1.67
ES 14/Flamura 85	TK (Eskisehir)	WW	1.67
Pedigree unknown	Unknown	WW	1.67
ORKINOS-1	TK (Ankara) – TCI	WW	1.67
BURBOT-6	OR – TCI	WW	1.67
QT9048/Cunningham	AUS – CIMMYT	SW	1.67
LOV41//LI7/LE2062	ARG – TCI	WW	2.00
F130L1.12/Attila	CIMMYT – TCI	WW	2.00
Pedigree unknown	Unknown	WW	2.00
Pedigree unknown	Unknown	WW	2.00
Altay 2000	TK (Eskisehir)	WW	2.00
Potch 92	South Africa	WW	2.00
Sunco/Pastor	AUS-CIMMYT	SW	2.00
KS82W409/SPN//CA8055	TCI	WW	2.33
PYN//TAM101/AMI/3/KRC66/SERI	TCI	WW	2.33
Nemura/KAUZ//AGRI/NAC	TCI	WW	2.33
HAWK/ARI	TK (Konya)	WW	2.33
TX71A1039-VI*3/AMI(TX81V6603)//MVR16-85	TK (Konya)	WW	2.33
Zargana-3	TCI	WW	2.33
Dagdas	TK (Konya)	WW	2.33
Suzen 97	TK (Eskisehir)	WW	2.33
Zargana-3	TCI	WW	2.33
Predela/Suzen	TK (Eskisehir)	WW	2.33
ES84-24/Dynasty	TK (Eskisehir)	WW	2.33
Sunco/2*Pastor	AUS – CIMMYT	SW	2.33
MVR27-82//LI7/LE2062	ARG – TCI	WW	2.33
Sunco/Pastor	AUS – CIMMYT	SW	2.33
Sunco/Pastor	AUS – CIMMYT	SW	2.33
Sunco/Pastor	AUS – CIMMYT	SW	2.33
Sunco/Pastor	AUS – CIMMYT	SW	2.33
Bolal 2973/Thunderbird	TK (Konya)	WW	2.53
Dachnaya/LAJ3302	CIMMYT-TCI	WW	2.67
Zargana-2	TCI	WW	2.67
ECVD12/KAUZ//Unknown	TCI	WW	2.67
OK81306/SITTA//AGRI/NAC	TCI	WW	2.67
OK81306//ANB/BUC/3/GRK/7C	TCI	WW	2.67
Cerco/Alondra	TK (Eskisehir)	WW	2.67
Gerek79	TK (Eskisehir)	WW	2.67
Katea-1	BUL – SAD	WW	2.67
JING411//PLK70/LIRA/3/GUN91	TCI	WW	2.67
Sunco/2*Pastor	AUS – CIMMYT	SW	2.67
Sunco.6	AUS – CIMMYT	SW	2.67
Sunco/Pastor	AUS – CIMMYT	SW	2.67
Sunco/Pastor	AUS – CIMMYT	SW	2.67
Sunco/Pastor	AUS – CIMMYT	SW	2.67
Sunco/Pastor	AUS – CIMMYT	SW	2.67
Sunco/Pastor	AUS – CIMMYT	SW	2.67
Sunco/Pastor	AUS – CIMMYT	SW	2.67
DF431.83/2*Rascon-37	CIMMYT	WD	2.67

Table 3 (continued). Germ plasm from replicated Root Rot Elite Line Nursery 0203 with field resistance to root rot complex under inoculated field conditions at Cumra, Turkey. Differences in cultivars are significant at 0.01; SED = 0.5.

Cross	Origin [1]	Type [2]	Score [3]
URA/YAZI-48	CIMMYT	WD	2.67
F12.71/SKA//FKG15/3/F483/4/CTK/VEE	TCI	WW	3.00
Orkinos-3	TK (Ankara) – TCI	WW	3.00
TAM200/KAUZ	CIMMYT – TCI	WW	3.00
PLK70/LIRA"S//30-KZ-1	TK (Konya)	WW	3.00
Sultan95	TCI	WW	3.00

[1] Origin is ARG = Argentina, AUS = Australia, BUL = Bulgaria, CIMMYT = CIMMYT–Mexico, OR = Oregon, U.S.A., SAD = Sadovia, TCI = Turkey/CIMMYT/ICARDA IWWIP, TK = Turkey National Program, and YA is unknown.

[2] WW = winter wheat, SW = spring wheat, WD = winter durum.

[3] Root rot score is defined as the percent of whiteheads in inoculated versus noninoculated plots and ranges from 0–5 where 0 = no symptoms, 1 = < 5 % whiteheads, 2 = 5–10 %, 3 = 10–30 %, 4 = 30–50 %, and 5 = > 50 % whiteheads.

Dryland root rot: a brief update of survey, tolerance, seed treatment, and micronutrient interaction in 2003–04.

A survey of over 450 plant samples from the plateau has implicated *F. culmorum* to be the main causal agent of dryland root rot. Inoculated yield trials within the mixture, *B. sorokiniana, F. culmorum* and *F. pseudograminearum*, caused yield losses up to 45 % in winter wheats. Decreasing tolerance was found, triticale > barley > bread wheat > durum wheat. Several tolerant lines were identified. Similarly, under inoculated-field conditions, 7,167 genotypes (lines/cultivar) of bread and durum wheat, barley, and triticale have been tested, with over 200 of these being promoted for further testing (as mentioned above), confirmation, and subsequent integration into the IWWIP. These lines also were sent to CIMMYT–Mexico for incorporation into spring wheat germ plasm. Seed treatment with different fungicides increased the yield compared with the control, however, their use is limited based on their economic feasibility. Application of the microelements of Zn and Fe were found to be highly important at reducing losses to dryland root rots, however, cultivar-specific reactions were evident. The current information reinforces the use of an IPM approach to these dryland root rots with an emphasis on host–plant resistance.

Capacity building for soilborne pathogen training.

The IWWIP is training Turkish scientists and scientists from the region in the field of soil disease cereal research. This training includes postgraduate training and special courses such as 1st International Soil Borne Pathogen training course conducted in Turkey in June 2003 under the coördination of CIMMYT pathologist Dr. Julie Nicol hosted by MARA Turkey. Twenty-five researchers from 13 countries took part in a dynamic, advanced course, from countries such as Afghanistan, Iran, Kazakhstan, Syria, Morocco, Tunisia, India, and Uzbekistan, and were guided by high-profile, experienced scientists from Australia, France, Turkey, CIMMYT, and ICARDA.

Participants were chosen for their perceived potential for using and disseminating in their home countries the knowledge acquired in the course. They were highly motivated, and it is hoped that they will contribute to tackling soilborne diseases in the future. The emphasis of this course was on providing practical solutions to real problems encountered in the participants' home countries. This course, mainly due to the diversity and motivation of the trainees and the commitment of the lecturers, has provided a basis for effective future collaboration on root and crown rot and nematodes. Already, over 13 special nurseries containing promising resistance sources for root rot and nematodes have been disseminated to participants from the course and an International Root Disease Network has been formed. We are highly grateful to the sponsors, principally lead by the ATSE Crawford Fund, CIMMYT, MARA, ICARDA, GRDC, ACIAR, and the Kirkhouse Trust. For further information regarding the course please contact Dr, Julie Nicol (j.nicol@cgiar.org).

Concluding remarks.

We believe that by conducting this highly focused, complex, and difficult research, we can clearly define the soilborne constraints in the winter wheat regions of CWANA and ultimately significantly improve wheat production and sustainability of the cropping systems in our region. The key to this will involve a breeding approach to produce high-yielding, quality, adapted germ plasm combined with multiple root disease resistances and microelement efficiencies, complemented with appropriate management practices. This work is large and encompassing, and we welcome collaboration from interested parties.

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ITEMS FROM THE UKRAINE

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Manifestation of Vrn gene effects in isogenic winter wheat lines.

V.V. Zhmurko, O.A. Avksentyeva, O.U. Gerashchenko, and A.F. Stelmakh.

Vrn genes control the type of development in wheat. In the dominant condition, these genes determine a spring type of the development. In the recessive condition, a winter type is formed. Research into the physiology and biochemical process of these lines may help us determine the nature of the regulation in wheat. Our research studies the possible effects of the *Vrn* genes on carbohydrate metabolism and oxidizing activity at the different *Vrn* loci in isogeneic lines. We used two cultivars of the soft wheat, Priboy and Mironovskaya 808, with one and two dominant *Vrn* genes for spring-type development. These lines were created by A.F. Stelmakh (1998).

Results of our research on development rate show that young spikes formed in lines with the dominant locus 22. In the Priboy lines, dominate locus 11 caused quickest transition to spike formation. The line with the dominant locus 33 formed spikes earlier than the line with locus 22, but later than with locus 11. In the Mironovskaya 808 line with locus 11 and 33, no difference in speed of transition to spike formation is found (Table 1).

Table 1. Rate of spike development in isogenic lines of wheats with *Vrn* genes. Data are for days until spikes were formed.

Vrn locus	Priboy	Mironovskaya 808
11	42 ± 3	42 ± 3
22	70 ± 3	70 ± 3
33	58 ± 2	38 ± 1
1122	44 ± 2	40 ± 2
1133	48 ± 2	55 ± 3
2233	53 ± 3	55 ± 2

Spike formation in the Priboy lines with two dominant loci 2233 was later than in plants with the loci combinations 1122 or 1133. Plants of Mironovskaya 808 with the two dominant loci 1133 or 2233 formed spikes later than those with the dominant locus combination 1122 (Table 1). Locus 22 is shown to be more effective.

Research on oxidizing activity showed that catalase-activity is lower and peroxidase-activity is higher in Priboy and Mironovskaya 808 lines with one dominant locus 22 than at lines with dominant locus 11 or 33 (Table 2).

Table 2. Oxidoreductase activity in leaves of isogenic lines of wheat by *Vrn* genes.

	Catalase (ml O_2/g[a]·min)		Peroxidase (E/g·sec)	
Vrn locus	Priboy	Mironovskaya 808	Priboy	Mironovskaya 808
11	149 ± 3	153 ± 3	11.6 ± 0.50	11.0 ± 0.23
22	141 ± 4	138 ± 1	17.7 ± 0.25	13.9 ± 0.51
33	152 ± 2	157 ± 3	15.1 ± 0.33	10.8 ± 0.47
1122	147 ± 2	149 ± 2	12.9 ± 0.40	12.3 ± 0.46
1133	152 ± 1	137 ± 1	12.2 ± 0.40	11.9 ± 0.27
2233	146 ± 2	136 ± 2	12.0 ± 0.42	11.1 ± 0.37

Catalase activity is higher in the Priboy line with two loci 1133, than in lines with loci combinations 1122 and 2233. The catalase activity in Mironovskaya 808 lines is higher with loci 1122, than in those with 1133 or 2233 (Table 2). Peroxidase activity in Priboy and Mironovskaya 808 with all two-loci genotypes were the same (Table 2).

We found an essential difference in oxidase activity in lines with one dominant locus. The greatest effect on vernalization from the dominant locus 22. This locus has a large effect on growth rate in our study and probably plays a part in grows rate regulation by determining metabolic processes, in particular oxidoreductase activity. Oxidase activity in lines with two dominant loci has the same effect as grows rate. Different *Vrn* loci determine growth rate through genetic control of oxidoreductive activity.

The dynamics of carbohydrates show that carbohydrate content in one and two-loci lines increases in leaves during the day (Table 3). Lines with one locus are different in carbohydrates accumulation; lines with locus 22 of both cultivars have a higher accumulation during the day than at lines with 11 and 33. This difference is not only because of the ability locus 22 to cause a greater accumulation, but also because lines 11 and 33 may have a more intensive flow of carbohydrates from leaves during the day. The same carbohydrate accumulation during the day was found in Mironovskaya 808 lines with two loci; more intensive in lines with 1133 than in lines with 1122 and 2233. In lines of Priboy with two *Vrn* loci, the higher accumulation was in lines with 2233. Lines 1122 and 2233 were identical.

Table 3. The daily dynamic content of reduced sugars in leaves of wheat isolines with different *Vrn* genes. Sugars (mg/g dry mass) were recorded at 8 a.m. (morning) and 6 p.m. (evening).

	Priboy			Mironovskaya 808		
Vrn locus	Morning	Evening	Accumulation	Morning	Evening	Accumulation
11	10.7 ± 0.41	13.0 ± 0.43	2.3	19.8 ± 1.22	19.8 ± 1.35	0.0
22	10.5 ± 0.32	19.1± 0.69	8.6	17.4 ± 1.05	33.8 ± 2.05	16.4
33	9.0 ± 0.21	13.3 ± 0.46	4.3	9.0 ± 0.53	17.3 ± 0.86	8.3
1122	6.9 ± 0.11	12.1 ± 0.27	5.2	16.1 ± 0.97	33.5 ±1.92	17.4
1133	16.1 ± 0.75	21.3 ± 0.92	5.2	14.3 ± 0.75	42.1 ± 2.54	27.8
2233	16.4 ± 0.68	31.3 ± 1.25	14.9	20.3 ± 1.10	38.7 ± 2.03	18.4

Our results show that lines with different *Vrn* gene and, therefore, growth rates, also differ in carbohydrate accumulation, establishing the connection between carbohydrate metabolism and grows rate. Our previous results show that unvernalized winter wheat with slow growth rates differ from vernalized winter wheat with quick growth rate by having a smaller accumulation and flow of carbohydrates (Tsybulko et al. 2000). We suggest that one mechanism that determines spring-type development of wheat is a change in the intensity of carbohydrates metabolism caused by *Vrn* genes.

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Inheritance of alien characteristics in hybrids between T. aestivum and wheat introgression lines.

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Introduction. Wild species of wheat and its relatives are known to be accessible sources of genes for use in wheat improvement, especially with respect to disease resistance, adaptation to harsh environmental factors, and seed quality. A number of wheat–alien introgression lines with high resistance to powdery mildew, leaf and stem rust, frost and heat tolerance, high protein content, and some morphological characters were developed from the wide cross 'triticale (8x) AD 825/*T. turdigum* subsp. *durum* cultivar Chernomor after spontaneous hybridization with collection strain H74/90-245 (Motsnyy et al. 2000a, b; 2002a, b). This study investigates plant viability, meiotic chromosome behavior, and inheritance of alien and wheat characteristics in hybrids obtained by crossing several introgression lines with a commercial bread wheat cultivar. The investigation was carried out within a program for the development of a genetic collection of bread wheat lines with qualitative characters.

Material and methods. The material included hybrids between the cultivar Odesskaya 267 and several introgression lines, between two introgression lines, and between Odesskaya 267 and the supposed parental (for mentioned lines) sib-strain H74/90-245. H74/90-245 was derived in Bulgaria from the cross 'synthetic ((*T. timopheevii* /*Ae. tauschii*)/Tom Pouce Blanc/Avrora/Rusalka)' and was received from Dr. Ivan Panayotov. In 2000, F_2 seed from the same families (offspring of the same F_1 plants not isolated) were separately sown in two different plots; Phito, a plant pathology plot with natural and artificial (leaf and stem rusts) infection pressure, and Field, a plot with natural infection only. There were different environmental conditions in the plots in 2000. The winter (frost) was harder in Field and autumn and spring droughts were present in Phito. In 2002 and 2003, the F_2 seed (from isolated F_1 plants) were sown only in Phito. In 2003, 60 kernels also were sown in Field. Too much water was received during the winter of 2002 and a hard frost in the winter and a strong spring drought were experienced in 2003.

Results and discussion. Some sterility was noted in the F_1 hybrids of 'Odesskaya 267/H74/90-245' cross. Seed set was 0–66 (average 38.0) seed/spike and 0–564 (average 147.1) seed/plant. F_2 seed germination was low (53.5–86.0 %), except in the cross '*Erythrospermum* 217/97-B/*Hostianum* 242/97-2-B' (91.4 %). A lethality factor is present in this material.

Meiotic observations revealed the presence of 19 ring bivalents (the maximum) plus univalents or rod bivalents in the F_1 of 'Odesskaya 267/H74/90-245' hybrids. For the introgression lines, two univalents or a rod bivalent were observed in 66.4 or 33.3 % of the PMCs in the F_1 hybrids of 'Odesskaya 267/*Erythrospermum* 200/97-2-B'and '*Hostianum* 242/97-1/Odesskaya 267', respectively. In '*Erythrospermum* 217/97-B/*Hostianum* 242/97-2-B' (some plants), 21 ring bivalents were observed in two (of 801 studied) PMCs. The normal constitution of $13^{II} + 9^{I}$ was usually observed in meiosis of the F_1 hybrids between the lines *Erythrospermum* 200/97-2-B, *Hostianum* 242/97-1', and *Hostianum* 242/97-2-B and *T. turgidum* subsp. *durum*. Therefore, a translocation or substituted alien chromosome is present in the A or B genome of the introgression lines. In *Erythrospermum* 217/97-B, the translocation (or substitution) is heterozygosis. Comparatively regular meiosis in some F_1 plants and good F_2-seed germination were observed in the '*Erythrospermum* 217/97-B/*Hostianum* 242/97-2-B' cross.

The data in the Tables 1 (p. 179) and 2 (p. 180) show different segregation in the various conditions. Only the hairy glume character was inherited as expected (one gene). For the alien characters, *Hl* (hairy leaf) is assumed to be one gene with different expression (concerning the lower part of the leaf blade) or two closely linked genes (Hl^{up} and Hl_{low}).

Table 1. The F_2 hybrid viability and segregation for crosses between wheat and alien introgression lines under various conditions. Phito was a plant pathology plot with high (natural + artificial) infection pressure from leaf and stem rusts and a strong drought in 2000. Field was a plot with natural infection and a strong frost in 2000. Characteristics include *Hl*, hairy leaf; *Rs*, rough stem, *Hg*, hairy glume; *Lr*, leaf rust resistance; *Sr*, stem rust resistance; and *Pm*, powdery mildew resistance.

Plot	Seed	Germination (%)	Wintered (%)	Saved (%)	Characteristic	Dominant:Recessive Observed	Dominant:Recessive Expected	X^2	% of dominants
2000 (hard winter, autumn and spring drought).									
Cross Odesskaya 267 / H74/90-245									
Phito	156	96	84	75	*Hl*	58:17	3:1	0.22	77.3
		(61.5)	(87.5)	(78.1)	*Rs*	61:14	13:3	0.00	81.3
				(89.3)	*Lr*	55:20	3:1	0.11	73.3
					Sr	72:3	15:1	0.65	96.0
					Pm	62:13	3:1	2.35	82.7
Field	340	218	?	113	*Hl*	65:48	3:1	18.4	57.5
		(64.1)		(62.4)	*Lr*	65:48	3:1	18.4	57.5
		181			*Pm*	90:23	3:1	1.30	79.6
2002 (soft winter, too much water in winter).									
Cross *Hostianum* 242/97-1 / Odesskaya 267									
Phito	286	153	109	73	*Hl*	55:18	3:1	0.00	75.3
		(53.5)	(71.2)	(47.7)	*Hg*	56:17	3:1	0.11	76.7
				(67.0)	*Lr*	37:36	9:7	0.92	50.7
					Sr	49:24	3:1	2.42	67.1
2003 (too hard frost in winter, spring and summer drought).									
Cross Odesskaya 267 / *Erythrospermum* 200/97-2-B									
Phito	?	231	99	65	*Hl*	34:31	3:1	17.9	52.3
		(86.0)	(42.9)	(28.1)	*Sr*	49:16	3:1	0.01	56.5
				(65.7)					
Field	60	44	25	23	*Hl*	13:10	3:1	4.19	56.5
		(73.3)	(56.8)	(52.3)					
				(92.0)					
Cross *Erythrospermum* 217/97-B / *Hostianum* 242/97-2-B									
Phito	152	139	109	96	*Hl*	62:34	3:1	5.56	64.5
		(91.4)	(78.4)	(69.1)	*Hg*	74:22	3:1	0.22	77.1
				(88.1)	*Lr*	65:20	3:1	0.10	76.5
					Sr	62:34	3:1	5.56	64.5

A somewhat hairy upper part of leaf blade of young leaves (III–IV) was observed in Odesskaya 267, which may sometimes distort segregation (especially in BC_1 populations). This character (as well as the others) was influenced by positive and/or negative natural selection and its penetrance is unknown.

In 2000, in families were selection was not an influence (≈ 100 % of saved F_2 plants), segregation was as follows: 24:11 ($X_{3:1}$ = 0.77 with regard to *Hl*), 30:5 ($X_{13:3}$ = 0.46 with regard to *Rs*), 25:10 ($X_{3:1}$ = 0.24 in regard to *Lr*), 32:3 ($X_{15:1}$ = 0.32 in regard to *Sr*), and 28:7 ($X_{3:1}$ = 0.47 in regard to *Pm*). Odesskaya 267 is known to contain ineffective genes *Lr3a, Sr5, Sr8a*, and *Sr36*, but does not contain any *Pm* genes (L.T. Babayants 2002, personal communication). However, in 2000–03, Odesskaya 267 was very susceptible to the studied diseases at the adult plant stage for the race population.

In 2002, no hard frost or drought were recorded in the autumn–spring period. Many F_2 seedlings died from too much water in winter and, probably, there was not any selection for or against alien characters. Explaining the disparity

Table 2. Backcross hybrid viability and segregation for crosses between wheat and alien introgression lines under various conditions. Phito was a plant pathology plot with high (natural + artificial) infection pressure from leaf and stem rusts and a strong drought in 2000. Field was a plot with natural infection and a strong frost in 2000. Characteristics include *Hl*, hairy leaf; *Rs*, rough stem, *Hg*, hairy glume; *Lr*, leaf rust resistance; *Sr*, stem rust resistance; and *Pm*, powdery mildew resistance.

Plot	Seed	Germination (%)	Wintered (%)	Saved (%)	Characteristic	Dominant:Recessive Observed	Dominant:Recessive Expected	X^2	% of dominants
2002 (soft winter, too much water in winter).									
BC_1 *Hostianum* 242/97-1 / Odesskaya 267									
Phito	62	47	24	19	*Hl*	12:7	1:1	1.32	63.2
		(75.8)	(51.1)	(40.4)	*Hg*	8:11	1:1	0.47	42.1
				(79.2)	*Lr*	14:5	3:1	0.02	73.7
					Sr	17:2	3:1	2.12	89.5
2003 (hard frost in winter, spring and summer drought).									
BC_1 Odesskaya 267 / *Erythrospermum* 200/97-2-B // Odesskaya 267									
Phito	57	52	35	28	*Hl*	15:12	1:1	0.33	55.6
		(91.2)	(67.3)	(53.8)	*Sr*	19:9	3:1	0.76	67.9
				(80.0)					
BC_2 *Hostianum* 242/97-1 / Odesskaya 267									
Phito	61	57	49	43	*Hl*	13:8	1:1	1.19	61.9
		(93.4)	(86.0)	(75.4)	*Sr*	37:6	3:1	2.80	86.0
				(87.8)					
F_2BC_3 (Odesskaya 267 / H74/90-245) self // Odesskaya 267									
Phito	92	85	67	64	*Hl*	14:19	1:1	6.25	65.6
		(92.4)	(78.8)	(75.3)#	*Sr*	48:15	3:1	0.05	76.2
				(95.5)					

between the F_2 and BC_1 segregations and the low frequency of F_2 dominant plants resistant to leaf and stem rusts is difficult. Some heterozygous F_2 plants may have been incorrectly classified as susceptible, because the plants were under the disease pressure from a race population and not a monoisolate.

A hard frost occurred in the winter of 2003. The low frequency of *Hl* and *Sr* in the F_2 plants may have been due to the selection against these characters. The low frequency of resistant F_2 plants also may be due to the presence only one *Sr* gene from the line H74/90-245 or any other *Sr* genes in the introgression lines.

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Wheats derived from crosses between triticale and bread wheat.

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The development of new, original wheats by genetic enrichment with rye genes will expedite the formation of hereditary variation that is the basis for effective breeding of the cultivars that meet modern demands. At the Plant Production Institute named after V.Ya. Yurjev (Ukraine), genetic selection on wheat is aimed at combining wheat and rye traits by means of creating and improving wheat–rye amphidiploids. Thus, making wide crosses between hexaploid forms of triticale and bread wheat becomes possible. Hybridizing triticale with wheat results in variation by means meiotic crossing over in A and B genomes of triticale and wheat. If we take into account the three species of triticale (by systematics of A.F. Shoulyndin), then such crosses result in greater possibilities for widening the gene pool of bread wheat. The original material for producing hybrids are wheat–rye forms or triticale and wheat genotypes having 1–2 pairs of rye chromosomes. Bread wheat with a reduced number of rye chromosomes as compared to hexaploid triticale, the maternal parent, are referred to as wheat revertants. Here we present generalized data on studies of divergent lines of winter bread wheat in subsequent generations.

A large, diverse number triticale forms and cultivars originating at our Institute and at a number of foreign research institutions (Russia, Poland, Germany, and Canada) and new cultivars of winter bread wheat were involved in our crosses. Winter, hexaploid triticales with different genomic structures were used as the maternal parents. Hybrid plants with a bread wheat type were identified in the crosses, with substituted triticale forms at 47 %, amphidiploid triticale with the entire rye genome at 44 %, triticales with rye introgressions at 7 %, and *Secale–Triticums* at 2 %.

When studying the generations of hybrids from F_3 to F_{10}, we observed a gradual increase in the number of morphologically homogeneous lines. By the F_{10}, 95 % of wheat revertant lines were phenotypically homogeneous. The ploidy stabilization (42-chromosomal level) in wheat revertant genotypes was elucidated in the F_5–F_6. The higher productive lines were obtained from crosses of triticale, A60, A73, A60/A206, Tarasivs'kyi, Grado, and the crosses Dons'ka polukarlik/Sarativs'ka 4//A206 and Skorospelka 4/Kharkivs'ka 55//A206/3/Dons'ka polukarlik with winter bread wheats Olvia, Albatros odess'kyi, Dons'ka polukarlik, Myronivs'ka ostista, Erythrospermum 8-88, Lutescence 1019-87, Byelotserkivs'ka jubileina, Samars'ka, and Ukriraceg.

Most of the wheat revertant lines obtained possessed a common morphotype inherent in *T. aestivum* species. But 6 % of the investigated lines differed somewhat in plant color and spike morphology. The blades and the spikes of those plants were dark green; the stalk with an anthocyanin color. The spike was long (up to 12 cm), fusiform, loose with rigid glumes, and fertile (39–51 kernels/spike) but poorly threshable. The grain was large, vitreous, elongated–oval, and frequently with a deep furrow. Disease resistance in these lines is combined with high winter hardiness. A cluster analysis of morphologically stable lines for five productivity traits (1996–2000) showed that these lines are close to the standard *T. aestivum* with T1B·1R substitution. Electrophoresis of storage proteins in these lines proved the presence of the T1B·1R translocation (Souvorova et al. 2000). The interesting fact is that such plants were developed from crosses with substituted and introgressive triticales.

Winter hardiness in the wheat revertant lines varied from 8–9 in years favorable to overwintering. During the severe winter of 2002–03 when 80 % of the winter bread wheat sown were killed, 50 % of our lines survived. Freezing in artificial climate-controlled chambers has confirmed their high winter hardiness. One remarkable property of the wheat revertants is their intensive growth at the start of spring. The duration of the vegetation period for most revertant lines is similar to that of Albatros odesskiy, and 39 % are early, booting 3–6 days earlier than the standard. The developed lines are short- (64–77 cm) to medium-stemmed (96–105 cm) with stiff nonlodging straw.

One disadvantage of the wheat revertants is an increase in the number of sterile spikelets. Among the great morphological diversity, we still managed to select the lines having well-filled spikes (42–51 grains/main spike versus 37 grains in the standard) (Table 1, p. 182).

Table 1. Characteristics of the productivity elements in wheat revertant lines, 1995–2000

No.	Cross combination	Characteristic					
				Main spike			
		Plant height (cm)	Number of spikes/ plant	Length (cm)	spikelets/ spike	Grain/ spike	1,000-kernel weight (g)
390	A60/Albatros odesskiy	76.5	3.7	9.1	16.3	42.2	43.5
394	A60/Zernogradka 6	92.8	5.3	11.3	18.9	50.5	42.4
454	A60/A206//Olvia	82.0	4.7	8.4	16.9	49.8	42.7
457	Skorospelka 4/Kharkivs'ka 55//A206/3/ Dons'ka p.k./4/Myronivs'ka ost/5/Myronivs'ka ost	63.2	5.3	8.4	16.9	40.0	48.7
458	Skorospelka 4/Kharkivs'ka 55//A206/3/ Dons'ka p.k./4/Myronivs'ka ost/5/Myronivs'ka ost	61.1	3.5	8.3	17.1	38.2	43.6
461	A73/Erythrospermum 8-88	69.8	5.2	10.1	18.0	40.9	42.5
464	Dons'ka p.k./Sarativs'ka 4//A206/3/ Myronivs'ka ost	78.3	3.1	8.7	16.2	42.8	37.8
466	Suvgen2/2*SFG//Ukriraceg	86.1	4.1	10.2	16.8	45.5	42.5
Check	Albatros odesskiy	80.9	4.8	10.3	20.0	37.2	38.7
Check	*T. aestivum* line 1546	98.2	4.9	10.9	18.2	45.0	44.3

In the grain quality laboratory at our institute, we studied the winter bread wheat collection from the NCPGRU, where a considerable number of the revertants have been included. According to 3-year data (Louchnoi VV 2000) along with the cultivars in question that were developed by traditional methods, we noted that RVS 461 has a high test weight, 820 g/l. Lines RVS 457 and RVS 458 combine high vitreousness (66 % and 73 %, respectively) with high protein content (15.3 % and 15.6 %, respectively) and the highest gluten content among the cultivars studied (33.9 % and 34.5 %, respectively).

Conclusion. Our analysis of wheat revertant lines showed that it is possible to create a valuable original material by distant hybridization of triticale with bread wheat for selection on yield capacity and grain technological qualities.

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Genetic resistance of winter and spring wheat to leaf diseases.

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Wheat ranks high among other agricultural crops in Ukraine, which is why much attention is given to increasing grain yield and quality. Diseases considerably reduce these traits; losses in grain yield are about 20 % annually. Thus, an intensive search for chemical, biological, and integrated methods of disease control are being made in most countries of the world. Creating and releasing disease resistant cultivars is the most economical, reasonable, and necessary method for combating wheat pests. Because of the use of disease-resistant cultivars, an increase in worldwide production of ~ 30 % is realized each year. In addition, creating resistant cultivars prevents the need for pesticide applications, which is of great importance to environment protection.

For breeding programs, a continued search for original material with resistance to leaf diseases a consistent is needed in different countries of the world. The wheat gene pool is the basis for the identification of these sources with their subsequent inculcation into breeding programs.

Using resistant lines as original breeding material and their hybridization with the best cultivars and breeding lines to increase productivity and further testing in the field has become a general breeding procedure. Selection of genetically diverse, resistant material on the basis of infection background is a very important aspect for breeding work as well. The use of these sources of resistance resulted in the creation and spread of the cultivars with resistance genes useful in one or more zones. At this point, the problem of genetic protection against harmful organisms is not considered alone because of the fast variation in the pathogens. For an effective solution, we need to find original material with reliable resistance to leaf disease pathogens. In our work, special emphasis is given to the infection background, which reveals sources of high resistance. Studying the characteristics of resistance are the final outcome in solving the problem of resistance in wheat breeding. This process is continuous and consistent, so that the breeding progress of resistant cultivars causes pathogens to greater activity. Thus, it is necessary to search for new sources of resistance in order to determine their genetic nature of resistance. Our research is aimed at tackling these problems.

Materials and methods. Studies on resistance to leaf diseases, powdery mildew, leaf rust, and Septoria, were made under field conditions in the infection nurseries of the Division of Plant Immunity. To control the quality of inoculation with pathogens and the conditions for disease progress and spread, we used susceptible cultivars as indicators disease that were chosen from earlier data. The susceptible cultivars were planted in the infection nurseries with every 20 samples. The experimental plots also were planted with winter and spring wheats susceptible to leaf diseases. For plant inoculations, we used both the population spore material and material of separate races and stocks. Variation in population and race virulence and monitoring the dynamics of new virulent biotypes were conducted with cultivar differentiators, sources of resistance, and regional and promising cultivars and breeding lines.

Artificial infections of wheat for resistance to pathogens of powdery mildew, rust, and Septoria included the diagnosis and study of the species composition of pathogens (Anonymous 1988, 1989; Babayantz et al. 1988; Afonskaya et al. 1998) and studies of the effective resistance genes using genetic collections of the forms (350 samples) with substantiated resistance to diseases (Anonymous 1988; McIntosh et al. 1998). The years of study were characterized by considerable climatic variation, especially for plant growth and the development of leaf disease in 1997 and 2000, increased moisture and moderate temperature and for severe drought during the entire vegetative period, and a low level of pathogen development in 1996, 1998, and 1999. The weather conditions in 2001 and 2002 years included increased moisture at the beginning of the growing season and at flowering and severe drought and intense heat during grain formation and filling. The maximum infection with pathogens is shown in Table 2.

Table 2. Maximum leaf disease infection on winter and spring wheats, 1996–2003, in test plots of the Plant Production Institute, Kharkiv, Ukraine. A — indicates that the degree of infection was is insufficient to differentiate resistance.

	Year							
Disease	1996	1997	1998	1999	2000	2001	2002	2003
Spring bread wheat.								
Powdery mildew	25	100	100	100	65	40	60	40
Brown rust	100	100	100	—	100	100	100	40
Septoria	—	100	65	—	100	65	40	53
Hard spring wheat.								
Powdery mildew	15	40	65	40	40	40	65	25
Brown rust	25	100	25	—	100	100	100	25
Septoria	-	100	25	—	65	40	35	57
Winter wheat.								
Powdery mildew	65	—	—	100	25	—	—	—
Brown rust	80	—	—	80	100	65	45	—
Septoria	65	100	60	25	100	65	40	—

Results and discussion. The total number of samples studied in artificial infections and provocative backgrounds between 1996–2003 was 1000 winter and spring wheats annually. Individual and group resistance to leaf diseases were noted.

Powdery mildew. This wheat disease is one of the most harmful diseases, leading to decreases in yield quality. In the years studied, powdery mildew was found in areas under both winter and spring wheat at a significant degree. The degree of infection in hard spring wheats was somewhat less than that in spring bread wheats. The maximum infection in susceptible winter wheat samples ranged from 25.0–100 %, in spring bread wheat from 25.0–100%, and in spring hard wheat from 15.0–65.0 % (Table 2).

According to Ivanchenco (2000), 15 races of the powdery mildew pathogen were registered in the eastern part of the Forest-Steppe of the Ukraine during 1996–98 (58, 61, 60, 66, 26, 27, 4, 2, 0, 1, 44, 80, 42, 51, and 15); seven races were not registered (X_7, X_8, X_{11}, X_{12}, X_{13}, X_{14}, and X_{16}). Races 58, 61, 66, 4, 0, 27, 80, 15, 2, X_8, X_7, X_{14}, X_{13}, and X_{11}, were present every year, whereas others were observed in separate years, race 44 was present in 1996 and 1998, race 51 in 1996 and 1997, race 60 only in 1997, and race 26 only in 1998. Races 58, 2, 4, and 61 were predominate every year. For the most part, these are known races that are spread in different regions of the Ukraine. Resistance to race 80 only was found in the cultivar Al'batros odes'kyi every year. This cultivar is widely planted in the Ukraine and is used in the pedigrees of a number of cultivars. Babayants and Smilyanets (1991) noted the considerable spread of the dominate race 58 in the eastern part of the Forest-Steppe of Ukraine in 1991 and consisted of 15.1–25.0 % of the pathogenic population in the southern Ukraine.

Wheat resistance to powdery mildew is controlled by genes *Pm1–Pm24* and temporary genes *ML-Ad*, *MLar*, *MLBr*, *MLd*, *Ml-Ga*, *mlre*, *PmTmb*, and *Mlxbd* (Wilson 1985; Somasco 1990; Ma and Hughes 1993; Huang et al. 2000). In the Ukraine, the most effective, independently acting genes are *Pm4a* and *Pm4b*, and *Pm2–Pm6*; which are virulent to 10 % of the races and biotypes. Other genes are effective in complex with the above-mentioned genes (Babayantz and Smilyanetz 1991).

Lisovyi and Bogdanovych (2001a and b) looked at race virulence in the Ukraine for resistance genes in cultivars and differentiators and showed that the total degree of virulence is not very high at present but an increase would considerably complicate the problem of wheat breeding for powdery mildew immunity. Therefore, original and breeding material of wheat should be selecting on immunity to the widespread race 58, the most virulent race 51, and the potentially dangerous races 42, 80, X_7, and X_{16}.

At the Plant Production Institute n.a. V.Ya. Yurjev, our work centers on controlling the resistance in spring bread wheats that were created with resistance to powdery mildew, which includes 13 cultivars with identified *Pm* genes. Some cultivars that were resistant earlier but had lost their resistance were Kadett, *Pm3d Pm4b*; Solo, *Pm4b Pm1 Pm2*; Turbo, *Pm3d Pm4b*. The gene complexes *Pm3d Pm4b* and *Pm1 Pm2 Pm4b* appeared to be ineffective in the region for protection of spring wheats to the powdery mildew pathogen Rabinovych and Afons'ka 1996; Chetvertakova and Dolfgova 1997).

At the Kharkivs'kyi Selection Centre, the effective gene complexes provide cultivars Planet, Havet, Sappo, and Walter (*Pm1 Pm2 Pm4b Pm9*) and Nemares (*Pm1 Pm2 Pm4b Pm6 Pm9*) with immunity to the pathogen. The Swedish cultivar Nemares has provided stable resistance to powdery mildew for 6 years. The gene complex *Pm1 Pm4b* in the spring wheat cultivar Rang from Sweden and an independently acting gene *Pm4b* in cultivars Moris and Halberd from Great Britain and Arkas from Germany conditioned a medium resistance to powdery mildew. Among the collection material were two other resistant cultivars (Solvent and Leopard) with unidentified genes of immunity to powdery mildew.

During 1996–2003, we studied resistance to powdery mildew of more than 1,000 samples of breeding material and 263 samples of collection material of winter wheat. The breeding material was obtained from the Winter Wheat Breeding Division to study resistance and four sources with individual resistance were identified, Lutescence 512-95, Erythrospermum 623-94, Erythrospermum 763-94, and Lutescence 133-98. We studied material from the National Centre for Plant Genetic Resources of Ukraine (NCPGRU) and found seven sources of resistance in Ukranian cultivars from the Genetics Institute Vympel odes'kyi, Dnipropetrovs'kyi, Luna 3, Victoriya; from the Myronivs'kyi Institute for Wheat, Myronivs'ka 65; and the Russian wheats Volz'ka 23 and Prybaikal's'ka. A number of winter wheat cultivars with stable resistance to powdery mildew and resistance to the other leaf diseases are presented in Table 3 (p. 185).

We studied 665 samples of the breeding and collection material of spring wheat for resistance to powdery mildew in 1996–2003; 402 samples of bread and 119 samples of hard wheat. None of the lines were immune. Among the spring hard wheats were 16 lines with resistance to powdery mildew in the previous years: 90-536, 90-558, 90-643, 91-252, 92-656, 92-771, 93-422, 93-548, 93-730, 93-777, 93-924, 94-59, 94-120, 94-355, 94-644, and 94-659.

The resistance to the disease pathogen was preserved in the cultivars of hard spring wheat of the Yur'ev Plant Production Institute Kharkivs'ka 15, Kharkivs'ka 21, Kharkivs'ka 25, Kharkivs'ka 27, Kharkivs'ka 29, Kharkivs'ka 31, and Kharkivs'ka 33. The resistance in the spring bread wheat Kharkivs'ka 28 (line 91-380) remains stable.

Among the accessions from the world gene pool, 10 sources of spring bread wheat were resistant, Kurs'ka 2038 and Solveig (Russian Federation); Saxanaand and Galan (Czech Republic); Rascan, Sampan, and Avans (England); Banty (Poland); and four hard spring wheats Dipper 'S', Silvertaie, Altar 84, Alt01, Frailecillo 2 (Mexico), and Kremniy (Russian Federation) (Table 4). The breeding lines and collected samples identified as sources of resistance are recommended for use in selection for resistance to powdery mildew in winter wheat.

Table 3. Characteristics of winter wheat samples with resistance to diseases between 1994–2002. In some years, low background infection was due to unfavorable climactic conditions (*). Some data are based on 1 ([1]) or 2-years ([2]) data.

Cultivar	Country of origin	Year	Infection score		
			Powdery mildew	Leaf rust	Septoria
Myrych	Ukraine	1995–2000	7[1]	7	6–7
Pam'yaty Fedyna	Russia	1995–1998	9[1]	5	5
Nemchynivs'ka 25	Russia	1995–2000	7[1]	6[2]	4
V-8[1]-2	Bulgaria	1994–1997	8	9	5–6
33-09-23	Bulgaria	1994–1998	9	9	5–6
MV 23-88	Hungary	1994–1997	9	9	5–6
D 13349/86	Germany	1994–1998	9	7–9	6–7
248-82	Czech Republic	1994–1999	9	7	6–7
SMH2530	Poland	1997–2002	7[2]	6[2]	6–8
SMH 2893	Poland	1997–2002	9*	4	6–7
TAM200	USA	1994–1997	9	7–9	3–6
Charmany	USA	1997–2001	7[2]	8[1]	6–7
Rod	USA	1998–2002	9*	6[1]	7–8
WRB 860365	USA	1998–2000	9*	6	6–7

Table 4. Characteristics of bread and hard spring wheat samples with resistance to diseases between 1994–2002.

Cultivar	Country of origin	Infection score		
		Powdery mildew	Leaf rust	Septoria
BREAD WHEAT.				
Kurs'ka 2038	Russia	7	5	5
Sampan	Great Britain	7	5	4
CA92750	Mexico	6	9	4
Copper	USA	3	9	3
HARD SPRING WHEAT.				
Leucurun	Russia			
LBG	Russia			
Kremniy	Russia			
Step 3	Russia			
Don Pedro 87	Spain			
Dipper 'S' CD82607	Mexico	7	9	5
Silvertaie CD66589	Mexico	7–9	8	3
Wagtail 'S' CD60280	Mexico	7	8–9	5
Greenshank 38	Mexico	5	7–9	6–7
Jova 1	Mexico	4	9	6–7
Altar 84 Alto1	Mexico	7–9	9	5–7
Frailecillo 2	Mexico	7–9	8–9	5–6
G>londrino	Mexico	3	9	7
Scooper	Mexico	5	9	4
Lican INIA	Chile	5	9	3
Dur Wheat L	India	6	9	3
Bishoftu	Ethiopia	5	9	3

Septoria resistance in wheat. Septoria infects leaves, leaf sheaths (*S. tritici*), and spikes (*S. nodorum*). The first three genes of resistance to Septoria were described in the cultivar Bulgaria 88 and two U.S. winter wheats Oasis and Sullivan (*Stb1*); in the Brazilian cultivars Veranopolis and Nova Prata (*Stb2*); and in the Israeli line Israel 493 (*Stb3*) (Wilson 1985). Somasco (1990) reported on *Stb4* in two old varieties from the Netherlands, Cleo and Tadoma, and in a new cultivar Tadinia. The *Stb5* gene from *Ae. tauschii* was used in breeding the line 'Chinese Spring 8/Synthetic 7D' and E.R. Sears' synthetic wheat (Arraiano 2001) and gene *Stb6* is in the cultivar Flame (Brading et al. 2001).

The first genes for resistance to Septoria, *Snb1* and *Snb2*, were identified in the cultivar Red Chief and line EES (Ma and Hughes 1993) (Table 5). Wheat Synthetic 5D and line 'Chinese Spring*7/Synthetic 5D' (Worland 1995) are the source of *Snb3*. *Triticum timopheevii* subsp. *timopheevii* is the source of the resistance gene with the temporary symbols *SnbTM*. The derivatives of *T. timopheevii* subsp. *timopheevii* are resistant to Septoria and include lines S3-6, S9-10, and S12-1 (Ma and Hughes 1993, 1995). The sources for these genes can be found in McIntosh et al. (1998, 2002).

Table 5. Genes for resistance to wheat leaf and spike Septoria. Information sources for 1979–2001 are compiled according to works by McIntosh et al. (1998, 2002) and a number of other publications Wilson 1983; Somasco 1990; Ma and Hughes 1993).

Resistance gene and chromosomal location		Test lines, cultivars and lines with identified genes for resistance	Source of resistance	Reference
To *Septoria tritici*.				
Stb1		Bulgaria 88, Oasis, Sullivan		Wilson 1985
Stb2		Nova Prata, Veranopolis		Wilson 1985
Stb3		Israel 493		Somaco 1990
Stb4		Cleo, Tanidia, Tadorna		
Stb5		Chinese Spring*8/Synthetic 7D, Sears' Synthetic	*Ae. tauschii*	Arraiano 2001
Stb6		Flame, Bezostaya, Hereward, Shafir, Vivat		Brading et al. 2001
Stb7	4AL	Estanzuela, Federal		McCartney et al 2002
Stb8	7BL	Synthetic hexaploid W7984 (parent of ITMI population)		Goodwin 2002
To *Septoria nodorum*.				
Snb1	3AL	Red Chief, EE8 *Snb2*		Ma and Hughes 1993
Snb2	2AL	EE8 *Snb1*		Ma and Hughes 1993
Snb3	5LD	Chinese Spring /Synthetic 5D	*Ae. tauschii*	Worland 1995
SntbTM		*T. timopheevii* subsp. *timopheevii*/2*Wakooma, T. Timophee-S3-6, S9-10, S12-1		Ma and Hughes 1993

Septoria is manifested as blotches on all aboveground organs of plants and during all phases of growth. The winter wheat cultivars studied in the Plant Immunity Division of the Yur'ev Plant Production Institute were considerable infected with *S. tritici* and *S. graminis* pathogens. A disease epidemic eliminated the need to establish an artificial infection. Maximum infection was up to 65–100 % (Table 5). In 1999, fungal pycnidia appeared in the first 10-day period of May on the lower leaves of plants and then the infection spread to the middle and upper leaves but the flag leaf was left undamaged because the pathogen was suppressed by a severe drought. Under drought conditions, we studied resistance in 1,077 breeding and 465 collected samples of winter wheat and 665 breeding and 521 collected samples of spring wheat.

In the competitive variety trials of the Winter Wheat Breeding Division, two lines were the most resistant to Septoria (scores of 6–7), Lutescens 159-95 and Erythrospermum224. Ten lines were classified as medium resistant (score 5). The rest of the breeding material in the nursery was susceptible to highly susceptible to Septoria (Afons'ka 1996). Among the accessions in the world winter wheat gene pool, we identified eight cultivars and lines with resistance to Septoria pathogens with scores of 6–8 in 1996 and 1997, Myronivs'ka 33, Myrych, Khvylya Ukrainian cultivars, Granada, Arina, Ikarus, Panda; Niclas from Germany; and TX90V8727 from the U.S. These lines are recommended to use as the initial material in wheat breeding for resistance to *S. tritici*. The cultivars Myrych and Myronivs'ka 33 were employed as the initial material to develop resistant hybrids of winter wheat.

Leaf rust of wheat. We studied resistance of cultivars and samples to leaf rust in winter and spring wheats by planting between experimental plots of cultivars that are highly susceptible to leaf rust. The years of the studies were unfavorable for growth and spread of leaf rust in winter wheat, although the maximum infection of plants on separate entries reached 100%. During the study of a composite of local population of leaf rust pathogens by means of the cultivars, differentiators, and isogenic lines of Thatcher wheat, we ascertained that race 77 was dominate in spring and winter wheat crops in the region. In our conditions, the most effective genes in winter wheat genotypes are *Lr10* and *Lr24* in a number of the cultivars from Great Britain; and U.S. and French cultivars possessing *Lr10*; TAM106, Rocky, Mit, Sanday, Arthur, Wakefield, and Norman; and *Lr24*, Collin, Mesa, and Vasko (Dolgova et al. 1996).

The most resistant and highly productive cultivars are Seward (*Lr10*), Centurk (*Lr10*), Thunderbird (*Lr1 Lr24*), and cultivars with a combination of genes *Lr10* and *Lr24*, i.e., Parker 76 and Abilene. Durable resistance to leaf rust pathogens also is found in the U.S. cultivar Oasis with the complex *Lr9 Lr11*.

The analysis of Lr lines indicated that genes *Lr9*, *Lr10*, *Lr24*, *Lr25*, *Lr29*, and *LrTR* maintained immunity in winter wheat cultivars to pathotypes of race 77 in the northeastern region. A medium level of resistance was found in plants with *Lr23* (score 5–9) and *Lr14b* (score 7). A majority of independently acting genes are not effective (*Lr1*, *Lr2a Lr2c*, *Lr3b*, *Lr3bg*, *Lr3ka*, *Lr11*, *Lr12*, *Lr13*, *Lr14a*, *Lr26*, *Lr28*, *Lr30*, *Lr32*, *Lr34*, and *LrEch*) (Afons'ka 1996; Lisovyi and Pavlyuk 2004).

Complete protection of spring wheat cultivars against leaf rust infection is provided by a group of genes; *Lr10 Lr34* in the cultivar Opata 85; *Lr23 Lr26* in the cultivar Glenson 81 *Lr13 Lr17 Lr26 Lr34* in the cultivar Cumpas 88; and *Lr13 Lr17 Lr27+Lr31* in the cultivar Anahuac 75. All of these cultivars are immune and come from Mexico where intensive selection for leaf rust resistance is being made and great successes have been achieved. A high level of protection is provided by *Lr10* as well in genotypes from the U.S.; James (*Lr2a Lr10*), Jon (*Lr2a Lr10*), and Wheaton (*Lr10 Lr3*); and also with the complex of genes *Lr1 Lr2a Lr12 Lr13* in the Canadian cultivar Benito. All of these cultivars and selected new resistant breeding lines and accessions from the world's gene pool are sources for immunity to leaf rust.

During the years of our studies, 1,299 cultivars and samples were tested for resistance of winter wheat to leaf rust pathogens. Among 1,136 genotypes of winter wheat breeding material, we identified seven sources of resistance (score 7–9); Lutescens 512-95, Erythrospermum 542-95, Erythrospermum 545-95, Erythrospermum 218-99, Lutescens 220-99, Erythrospermum 238-99, and Erythrospermum 293-99, which remained resistant to the disease pathogene after 3 years of study. Among 163 genotypes of collected material, 62 cultivars showed high and medium levels of resistance to for 2–3 years of data. The best lines were Odes'ka 161 (Ukraine) and Keiser, Scout 66, and Lot W 8-769 (U.S.A.). IN 2002, 1-year immunological estimates identified 57 cultivars with high resistance to the disease. Cultivars of Ukrainian selection, Myronivs'ka 33 and Nosivchanka, are especially interesting and the resistance genes in these cultivars will be studied in the forthcoming years.

In spring wheat crops, we assessed the resistance to a leaf rust pathogen in 665 breeding and 521 collected samples. According to the 2–3 year studies, the best lines of hard spring wheat with resistance scores of 6–7 were 90-558, 90-1066, 90-1066a, 93-422, 93-548, and 93-607. These lines also are resistant to powdery mildew. According to our 4–5 year studies, two lines possessed resistance to both leaf rust and powdery mildew; 89-1520 and 91-380.

One hundred nineteen accessions from the world collection of hard spring wheat passed the trials, and 24 were resistant. The largest number 58.3 %, were cultivars from Mexico, which showed stable resistance to leaf rust for 3 years. From the spring bread wheat collection we identified 25 resistant lines, a majority of these lines also were Mexican cultivars including Alondra, CM83398, and Chirya; two cultivars from Africa, IP 11567 Karee and IP11571 Mopolo; and two cultivars from the Russian Federation, IP 11930 Cardinal and IP 11948 Obs'ka 44.

Our analysis of a collection for new sources and donors of resistance to leaf diseases of winter and spring wheat is ongoing. We are monitoring the race composition of the local pathogen populations. We also have formed a collection of the cultivars with the known resistance genes and have identified the sources of resistance and made recommendations for use in breeding work.

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The role of Ukrainian wheats in the pedigree of cultivars created at the North-Donyetskaya Experimental Agricultural Station.

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The North-Donyetskaya State Experimental Station is one of breeding institutions successfully working in the southern region of the Russian Federation. Winter wheat cultivars form our institute are grown in the Central Black-Soil, North-Caucasian, and Low-Volga regions of the Russian Federation and in Steppe region of the Ukraine, because they are well-adapted to steppe and southern forest–steppe conditions. Cultivars, year of release, HMW-glutenin composition, quality score, and region of cultivation of North-Donyetsk wheats in the Russian Federation and their pedigrees are given in Table 6 (p. 189).

The first cultivars from the Station, Severodonskaya and Tarasovskaya 29, were breed jointly with the All-Russian Science-Research Institution of Sorgo and other Grain Crops (Zernograd Breeding Centre). These cultivars were released in 1977 and in 1981, respectively. Severodonskaya has Myronivs'ka 808 in its pedigree, and Tarasovskaya 29 has Myronivs'ka 264. Since the release of these cultivars, wheat cultivars always have Ukrainian wheats in their geneology. In particular, wheats from Ukrainian institutions from the Forest-Steppe region, including the Institute of

Table 6. Winter bread wheat cultivar created at the North-Donyetsk State Experimental Agricultural Station 1977–2003 that are included in the State Registry of Breeding Achievement. High-molecular-weight glutenin subunit composition and quality score according to Payne (1987). Country of origin and quality scores of wheats involved in pedigrees are in parentheses; ITA = Italy, GER + Germany, MOL = Moldovia, RUS = Russian Federation, UKR = Ukraine, and YUG = Yugoslavia. Region of cultivation includes the Central Black-Soil (5), North-Caucasian (6), and Low-Volga (8) regions of the Russian Federation and in Steppe region of the Ukraine

Year of release	Cultivar and pedigree	HMW-glutenin subunits			Quality score	Region of cultivation
		Glu-1A	*Glu-1B*	*Glu-1D*		
1977	Severodonskaya Bezostaya 1 (RUS, 9) (a descendant of Ukrainian wheats Lutescens 17 and Ukrainka (9))/ Myronivs'ka 808 (UKR, 9)	1	7+9	5+10	9	—
1981	Tarasovskaya 29 Myronivs'ka Yuvilejna (UKR, 9)/Rostovchanka (RUS, 9) (a progeny of Myronivs'ka 264 (9))	1/2*	7+9	5+10	9	5, 6
1991	Severodonskaya 5 Tarasovskaya 29 (RUS, 9) (a derivative of the Ukrainian wheats Myronivs'ka 264 and Myronivs'ka 808)/Bilotserkivs'ka 47 (UKR)	2*	7+9	5+10	9	6
1992	Tarasovskaya 87 Dniprovs'ka 41 (UKR)/Donets'ka 5 (UKR, 9)	1	7+8/7+9	5+10	9.5	6
1996	Severodonskaya 12 Tarasovskaya 29 (RUS, 9)/Zaporizhs'ka ostysta (UKR)	2*	7+9	5+10	9	6
2000	Tarasovskaya ostystaya Tarasovskaya 29/Drina (YUG, 8)/Al'batros odes'kyj (UKR, 10) (a derivative of three Ukrainian wheats Selena, Majak, and Promin' (9) and in pedigree of which are present the Ukrainian cultivars Krymka, Odes'ka 12 (9.5), Odes'ka 16 (9), Yuzhnoukrainka, Odes'ka 51 (9.5), Prybiy (9), and Dniprovs'ka 521)	1	7+9	5+10	9	6
2001	Prestizh A selection from KS54104-1764 (USA)/3/Sava (YUG, 8)/Severodonskaya (RUS, 9)//Urozhajnaya (RUS, 9) (a descendant of the Ukrainian wheats Krymka and Odes'ka 3 (9.5))/4/Al'batros odes'kyj	1	7+8	5+10	10	5, 6
2001	Rosinka Tarasovskaya Soratnitsa (RUS, 9) (a progeny of the Ukrainian cultivars Odes'ka 66 (9), Odes'ka 51 (9.5), Odes'ka 16, and Ukrainka)/Donshchina (RUS, 9) (in the pedigree Ukrainian wheats Ukrainka, Odes'ka 3 (9.5), Odes'ka 16, and Myronivs'ka 808)	N	7+9	5+10	7	6, 8
2001	Tarasovskaya 97 Beltchanka 5 (MLD, 10) (a derivative of the Ukrainian cultivars Odes'ka 51 and Odes'ka napivkarlykova (9))/Spartanka (RUS, 9) (a descendant of the Ukrainian wheats Kharkivs'ka (LV), Krymka, Ukrainka, Lutescens 17, Myronivs'ka 264, and Myronivs'ka 808)	2*/N	7+8	5+10	9	5
2003	Rodnik Tarasovskyj Partizanka (YUG, 9) (pedigree: Bezostaya 1 (RUS, 9)/NS 116 (YUG, pedigree: Campodoro (ITA) /Heine VII (GER))/Zirka (UKR, 9.5)//Bilotserkivs'ka 18 (UKR)/Zirka (Odes'ka 16/Chhoti Lerma, IND)/Donskaya Yubilejnaya (RUS, 9) (Ukrainian wheat present in the progeny include Ukrainka (Lutescens 17 (five times)), Myronivs'ka 264 (this cultivar, from V.M. Remeslo, was breed from the spring durum wheat Narodna), Myronivs'ka 808 (twice) (breed from the spring wheat Artemivka), and Krymka (also called Turkey, twice, TUR (8.5), Odes'ka 3, and Odes'ka 16)	1	7+9	5+10	9	6
2003	Severodonetskaya Yubileynaya Tarasovskaya 29 (RUS, 9) (a derivative of Ukrainian wheats Ukrainka, Lutescens 17, and Myronivs'ka 808/Drina (Fortunato (ITA)/Redcoat (USA) (a progeny of Ukrainian wheats Red Fife (spring type, 9) and Krymka//Krasnodarskaya 57 (RUS, 9.5)/3/Al'batros Odes'kyj (UKR, 10) (a descendant of three Ukrainian cultivars Selena, Majak, and Promin', the pedigrees of these wheats include the six Ukrainian cultivars Krymka, Odes'ka 16, Odes'ka 22, Yuzhnoukrainka, Odes'ka 51, and Prybiy (9).	1	7+8/7+9	5+10	9.5	6

Plant Production n.a. V.Ya. Yurjev, Kharkivs'ka 63 (1969), Kharkivs'ka 82 (1981), and the spring durum wheat Narodna (1947); the Mironivs'kyi Institute of Wheat n.a. V.M. Remeslo, Ukrainka (1929), Myronivs'ka 264 (1960), Myronivs'ka 808 (1963), Myronivs'ka Yuvileyna (1971); Bilotserkivs'ka Experimental Breeding Station, Bilotserkivs'ka 47 (1981), Bilotserkivs'ka 18 (1982); Verkhnyachs'ka Experimental Breeding Station, Lutescens 17 (1940, the maternal form of the celebrated Russian cultivar Bezostaya 1 (1959)); and also wheats of Ukrainian institutions from Steppe region, Institute of Grain Farming (former All-Union Institute of Maize in the town Dnipropetrovsk), Dniprovs'ka 521 (1972) and Dniprovs'ka 41 (1977); the Donyets'k Institute of Agroindustrial Production, Donyets'ka 5 (1982) and the spring wheat Artemivka (1945); the Zaporizhs'ka Agricultural Experimental Station, Zaporizhs'ka ostysta (1980); the Breeding and Genetic Institute (Odessa), Odes'ka 3 (1938), Odes'ka 12 (1947), Odes'ka 16 (1953), Odes'ka 22 (1960s), Odes'ka 51 (1969), Yuzhnoukrainka (1971), Prybiy (1973), Odes'ka 66 (1979), Odes'ka napivkarlykova (1980), Zirka (1984), Mayak (1977), Promin' (1978), and Selena (1979). These last three varieties are parental forms of Al'batros Odes'kyj (1990), which, in its turn, is a parental form for new derivatives, including the cultivar Ukrainka odes'ka (1995).

New cultivars that passed into the state trials since 2003 include

Avgusta Pedigree: Albatros odes'kyj (UKR, 10) (Selena/Mayak/Promin' (9), all three from the Ukraine)/ Kharkivs'ka 82 (UKR, 9) (a selection from Kharkivs'ka 63 (UKR, 9) (Bezostaya 1 (RUS, 9)/Myronivs'ka 808 (UKR, 9)/Ukrainka odes'ka (UKR, 10) (a selection from Al'batros odes'kyj

Arfa Severodonskaya 12 (RUS, 9) (Tarasovskaya 29 (ROS, 9) (Myronivs'ka Yuvileyna (UKR, 9)/ Rostovchanka (RUS, 9) (Myronivs'ka 264 (UKR, 9) in pedigree)/Zaporizs'ka ostysta (UKR) (Skorospelka L 1 (RUS, 6)//Bezostaya 1 (RUS, 9)/Verkhnjachs'ka bezosta (UKR)/3/Bezostaya 1/Myronivs'ka 808 (UKR, 9)/Al'batros odes'kyj (UKR, 10).